

FINAL REPORT

Parallel In Situ Screening of Remediation Strategies
for Improved Decision Making, Remedial Design, and Cost
Savings

ESTCP Project ER-200914

February 2013

Rolf Halden
Kristen McClellan
Tomasz Kalinowski
Arizona State University

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CONTENTS

EXECUTIVE SUMMARY	1
1.0 INTRODUCTION	3
1.1 BACKGROUND	3
1.2 OBJECTIVE OF THE DEMONSTRATION	5
1.3 REGULATORY DRIVERS	5
2.0 TECHNOLOGY	9
2.1 TECHNOLOGY DESCRIPTION	9
2.2 TECHNOLOGY DEVELOPMENT	14
2.3 ADVANTAGES, RISKS AND LIMITATIONS OF THE TECHNOLOGY	16
3.0 PERFORMANCE OBJECTIVES	22
3.1 PERFORMANCE OBJECTIVE: Demonstrate capability of conducting mutually exclusive experiments in the same well	23
3.1.1 Data Requirements	23
3.1.2 Success Criteria	23
3.2 PERFORMANCE OBJECTIVE: No residue released into monitoring well during testing	23
3.2.1 Data Requirements	23
3.2.2 Success Criteria	24
3.3 PERFORMANCE OBJECTIVE: Determine potential side effects of remediation strategies	24
3.3.1 Data Requirements	24
3.3.2 Success Criteria	24
3.4 PERFORMANCE OBJECTIVES: ISMA study is cost-effective compared to a lab study of comparable scope	24
3.4.1 Data Requirements	25
3.4.2 Success Criteria	25
3.5 PERFORMANCE OBJECTIVES: Compare cost of conducting ISMA study vs. field trial	25
3.5.1 Data Requirements	25
3.5.2 Success Criteria	25
3.6 PERFORMANCE OBJECTIVE: Reproduce outcome of prior lab studies in the ISMA	25
3.6.1 Data requirements	25
3.6.2 Success criteria	26
3.7 PERFORMANCE OBJECTIVE: Reproduce outcome of prior field trials in the ISMA	26
3.7.1 Data requirements	26
3.7.2 Success criteria	26
4.0 SITE DESCRIPTION	27
4.1 Site Selection	27
4.2 Site Location and History	27
4.2.1 NAS North Island, San Diego CA – OU-20	27
4.2.2 Industrial Site, Mesa, AZ	30
4.3 Site Geology and Hydrogeology	31
4.3.1 NAS North Island – OU-20	31

4.3.2	Industrial Site, Mesa, AZ.....	31
4.4	Contaminant Distribution.....	32
4.4.1	NAS North Island – OU 20.....	32
4.4.2	Industrial Site, Mesa, AZ.....	34
5.0	TEST DESIGN	36
5.1	CONCEPTUAL EXPERIMENTAL DESIGN.....	36
5.2	BASELINE CHARACTERIZATION.....	37
5.2.1	NAS North Island - Prior Laboratory Treatability Studies	37
5.2.2	NAS North Island – Prior Field-Scale Pilot Study	38
5.2.3	Industrial Site – Prior Lab Treatability Studies	39
5.2.4	Water Sampling	41
5.3	TREATABILITY OR LABORATORY STUDY RESULTS	41
5.3.1	NAS North Island - Laboratory Flow-through Experiments	41
5.3.2	Industrial Site - Laboratory Experiments in Batch	46
5.3.3	Industrial Site - Laboratory Experiments in Flow-through Columns	47
5.4	DESIGN AND LAYOUT OF TECHNOLOGY COMPONENTS	51
5.4.1	Outer Shell of the ISMA	51
5.4.2	Pump Design.....	51
5.4.3	Sediment Column Tests	55
5.4.4	Delivery of Treatment Agent	56
5.4.5	Effluent Capturing	57
5.4.6	Handling of ISMA device	58
5.4.7	Power supply.....	60
5.4.8	Control during operation.....	61
5.5	FIELD TESTING.....	62
5.6	SAMPLING METHODS	63
5.6.1	DATA ANALYSIS.....	66
5.7	SAMPLING RESULTS	67
5.7.1	NAS North Island – ISMA Results.....	67
5.7.2	Industrial Site – ISMA Results	75
6.0	PERFORMANCE ASSESSMENT	78
6.1	PERFORMANCE OBJECTIVE: Demonstrate capability of conducting mutually exclusive experiments in the same well.....	79
6.2	PERFORMANCE OBJECTIVE: No residue released into monitoring well during testing	80
6.3	PERFORMANCE OBJECTIVE: Determine potential side effects of remediation strategies	82
6.4	PERFORMANCE OBJECTIVES: ISMA study is cost-effective compared to a lab study of comparable scope	82
6.5	PERFORMANCE OBJECTIVES: Compare cost of conducting ISMA study vs. field trial.....	82
6.6	PERFORMANCE OBJECTIVE: Reproduce outcome of prior lab studies in the ISMA	83
6.6.1	NAS North Island	83
6.6.2	Industrial Site - Degradation Rate Calculation	86
6.7	PERFORMANCE OBJECTIVE: Reproduce outcome of prior field trials in the ISMA	87
7.0	COST ASSESSMENT	88

7.1 Cost Model.....	88
7.2 Cost Drivers.....	91
7.3 Cost Analysis.....	92
8.0 IMPLEMENTATION ISSUES	96
9.0 REFERENCES	97
10.0 APPENDICES	101
10.1 Appendix A: Points of Contact.....	101
10.2 Appendix B: Synthetic groundwater recipe.....	102
10.3 Appendix C: ISMA checklists.....	103

LIST OF TABLES

Table 2–1. Overview of principal advantages and limitations of ISMA technology.....	21
Table 3–1. Performance Objectives as stated in the Demonstration Plan.....	22
Table 4–1. Monitoring well data at Site 2.....	35
Table 5–1. Experimental plan for NAS North Island.....	37
Table 5–2. Experimental plan for Site 2	37
Table 5–3. Experimental plan for Geosyntec laboratory batch study.....	40
Table 5–4. Power requirements of ISMA components and power provided by battery setup.	60
Table 5–5. Schedule of deployment activities.	62
Table 5–6. NAS North Island - Sampling Plan.....	63
Table 5–7. Industrial Site - Sampling Plan	64
Table 5–8. NAS North Island - Analytical Methods.....	65
Table 5–9. Industrial Site – Analytical Methods.....	65
Table 5–10. Groundwater collected during ISMA incubation.....	68
Table 5–11. Characteristics of groundwater quality parameters determined pre- and post deployment of the ISMA.	73
Table 6–1. Performance Objectives.	78
Table 6–2. TCE: calculated first-order degradation constants ($k \text{ day}^{-1}$).	83
Table 6–3. Cr(VI): calculated first-order degradation constants ($k \text{ day}^{-1}$).	83
Table 6–4. Variable Sourcing for Rate Calculations at NAS-NI.	85
Table 6–5. Overview of first-order reduction rates for Site 2. Results from lab and field experiments are listed.	87
Table 7–1. Direct material costs incurred during NAS-NI deployment.	88
Table 7–2. Direct costs for sample analyses incurred during NAS-NI deployment.	89
Table 7–3. Projected labor needs for future deployments.....	90
Table 7–4: Projected ISMA costs.	92
Table 7–5. Feasibility study project costs: OU-20, NAS-NI.	94
Table 10-1. Packing list for ISMA field deployments.....	104
Table 10.2. Failure modes and effects analysis for ISMA.....	105

LIST OF FIGURES

Figure 2-1. Schematic showing the <i>in situ</i> microcosm array (ISMA) apparatus suspended in a standard 4-inch (10 cm) inner diameter groundwater monitoring well. The device is supported from the surface via an umbilical tether, which holds it in place and provides power and control over the integrated multi-channel groundwater pumps. While deployed, desired test supplements (e.g., carbon sources promoting degradation of chloroethene), can be injected into sediment columns via the injection module, where they are allowed to interact with site groundwater and suspended microorganisms delivered by the peristaltic pumps. Upon retrieval, the contents of the apparatus can be analyzed (column effluent stored in effluent vessels; column sediment in microcosm array) to inform on processes that took place during <i>in situ</i> incubation	12
Figure 2-2. System components of an early embodiment of the <i>in situ</i> microcosm array (ISMA), showing a microcosm array that was based on a 96-well microtiter plate format.....	15
Figure 2-3. Cutaway view of the closure mechanism and the microcosm array of the Prototype ISMA (see text for details).....	15
Figure 4-1. Regional Location Map of NAS-NI.....	28
Figure 4-2. Site location map – OU 20.....	29
Figure 4-3. Site Map – Industrial Site.....	30
Figure 4-4. OU 20 TCE and Cr(VI) Plumes at OU-20.....	33
Figure 4-5. Perchlorate plume at Site 2.....	35
Figure 5-1. Schematic of laboratory column setup.....	42
Figure 5-2. Columns filled with sediment from NAS-NI being operated under continuous flow conditions in the laboratory. Entire assembly takes up approximately 5 sq. ft. in a fume hood. .	44
Figure 5-3. Close up view of the sediment columns and the Injection Module shown in Figure 5-2.	45
Figure 5-4. Results from column effluent samples.....	46
Figure 5-5. Biostimulation of site sediment (well HPA-1) and site groundwater in batch bottles with ethyl lactate as carbon source.	47
Figure 5-6. Concentration of perchlorate in column effluent over the course of the experiment. All experiments were conducted in triplicate, except influent concentration is measured from one sample at a time. Error bars represent standard deviation.	49
Figure 5-7. Results of quantitative PCR targeting the 16S rRNA gene of general bacteria and perchlorate reductase (<i>pcrA</i>). Shown are sediment results for the influent section of each column, which contained the highest numbers of bacteria compared to mid and effluent section. N/A = not tested in field experiment. Error bars represent standard deviation.	50
Figure 5-8. Detailed schematic of the ISMA device; Detail A - microcosm array; Detail B - injection module; Detail C - peristaltic pump.....	52
Figure 5-9. Performance control experiments: A - Pump flow rate accuracy for peristaltic benchtop pump (Ismatec Reglo Digital) and customized peristaltic pump used inside the <i>in situ</i> microcosm array (ISMA). Tests were conducted for 24 or 12 channels, respectively. B - Flow rate reproducibility between 12 channels for customized pump in the ISMA. Flow rates [μ L/min] were set to 20 (Δ), 50 (x), 100 (\square) and 200 (\circ) as indicated by the solid lines. Shown is the average of three measurements.....	54
Figure 5-10. Conservative tracer curves showing the bromide concentration in column effluent over time after a one-time injection of bromide. Sediment columns were filled with fine (<0.5	

mm – blue curve) or coarse (0.5 – 1 mm – red curve) grains. Experiments were conducted in triplicate.	56
Figure 5-11. Results from preservative test showing plate counts of column effluent treated with preservative and without (control). Experiments were conducted in duplicate; plating was done in triplicate. ND = non-detect (<300 CFU/mL).	58
Figure 5-12. ISMA section being handled by field worker and lowered into deployment well using a boom truck.	59
Figure 5-13. Securing clamp designed for use with the ISMA device. Left: top view; Right: side view.	59
Figure 5-14. Left: pre-assembled sections equipped with multi-channel quick connectors for fluid and electrical lines; Middle: connection of multi-channel quick in the field; Right: Load-bearing, waterproof connections are made between the different sections.	60
Figure 5-15. Solar panels and battery array used to provide electrical power for the ISMA device during deployment in remote locations.	61
Figure 5-16. Hexavalent Chromium detected in ISMA effluent post <i>in situ</i> incubation.	68
Figure 5-17. Chlorinated ethenes and ethene detected in ISMA effluent post <i>in situ</i> incubation.	69
Figure 5-18. Column pore water analysis for chlorinated ethenes and ethene after retrieval and again after 25 days with no flow. Increased concentrations of TCE dechlorination products in all the columns indicate that columns were still biologically active and dechlorinating after <i>in situ</i> incubation and exposure to Cr(VI).	71
Figure 5-19. Concentrations of primary contaminants detected in the ISMA deployment well compared to those found in ISMA bypass channels.	72
Figure 5-20. Sum of chlorinated ethenes (TCE, cDCE, and VC) detected in different sample types. Sum of chlorinated ethenes (TCE, cDCE, and VC) detected in the different samples types. Results indicate that columns were exposed to those concentrations of volatile organics found in grab samples of the groundwater and that the lower concentrations observed in captured effluent are a result of losses due to extended effluent storage in the ISMA.	73
Figure 5-21. Concentration of perchlorate in different experimental groups normalized to influent (bypass). Data represent composite samples collected over 21 days, representing the whole duration of the experiments. N/A = not tested in field experiment. Error bars represent standard deviation.	75
Figure 5-22. Relative bacteria distribution in sediment columns. Results of quantitative PCR targeting the 16S rRNA gene of general bacteria and perchlorate reductase (<i>pcrA</i>). Shown are relative results for all column sections.	77
Figure 6-1. Anion concentrations in column effluent of different experimental groups. Values represent the average of triplicates; error bars represent one standard deviation. Nitrate concentrations are corrected by subtracting the nitrate contribution from the added preservative.	80
Figure 6-2. Groundwater chemistry in well grab samples before and after ISMA deployment at site 1. Change in Concentration after ISMA Deployment represented as C/C ₀ . Fluctuations observed are due to tidal influence, additional parameters presented in Table 5-11.	81
Figure 6-3. Groundwater chemistry in well grab samples before and after ISMA deployment at site 2.	81
Figure 6-4. Acetone concentrations detected in ISMA effluent after incubation <i>in situ</i> at NAS-NI.	82

Figure 6-5. 2-Butanone concentrations detected in ISMA effluent after incubation <i>in situ</i> at NAS-NI	83
Figure 6-6. Sampling results from a batch bottle microcosm study performed by SiRem, demonstrating the effectiveness of bioaugmentation with KB-1® for treatment of groundwater from OU-20 at NAS-NI	86
Figure 7-1. Cost comparison of treatability study methods.....	96

LIST OF ACRONYMS

AC	Alternating Current
ASU	Arizona State University
AZ	Arizona
CA	California
cDCE	cis-Dichloroethene
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFU	Colony Forming Unit
DC	Direct Current
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
FMEA	Failure Modes and Effects Analysis
FTE	Full-time Employment
HS-SPME GC-FID	Head Space Solid Phase Microextraction Gas Chromatography Flame Ionization Detection
IC	Ion Chromatography
ID	Inner Diameter
ISMA	<i>In situ</i> Microcosm Array
ISW	In Situ Well Technologies, LLC
ITRC	Interstate Technology & Regulatory Council
LAU	Lower Alluvial Unit
MALDI-TOF-MS	Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry
MAU	Middle Alluvial Unit
MCL	Maximum Contaminant Level
MNA	Monitored Natural Attenuation
MW	Monitoring Well
NAS-NI	Naval Air Station North Island
NIH	National Institutes of Health
NRC	National Research Council
OD	Outer Diameter
ORP	Oxidation-Reduction Potential
OU	Operable Unit
PCR	Polymerase Chain Reaction
PEW	Persulfate Extraction Well
PVDF	Polyvinylidene Difluoride
RODD	Radius of Donor Delivery
ROI	Radius of Influence
RT	Residence Time
SRS	Slow-release Substrate
TCE	Trichloroethene

TDS	Total Dissolved Solids
TEDCO	Technology Development Corporation
UAU	Upper Alluvial Unit
UST	Underground Storage Tank
VC	Vinyl Chloride
WBO	Water Bore-Out

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EXECUTIVE SUMMARY

This report summarizes the development and demonstration of a new tool for remedial design, the *In Situ* Microcosm Array. It may serve potential end users as a general guide on how to utilize the ISMA technology in the design and interpretation of *in situ* feasibility studies.

Problem Statement. Before *in situ* remediation can be implemented at a hazardous waste site, bench-scale or field-scale feasibility studies are required. These are typically conducted in static batch-bottle microcosms, while an alternative approach, continuous-flow column studies, are rare in the remediation industry. Although scientifically constituting the “gold standard” approach to studying transport and reaction phenomena in saturated media, column studies are avoided due to a combination of factors including: considerable costs; complexity and difficulty in performing multiple replicates; and the requirement of considerable operator time. Although batch bottle tests may be adequate for qualitative screening of remedial design options, they are generally considered to have poor quantitative predictive power. In contrast, column studies are expected to produce both reliable qualitative and quantitative data, as they create a more realistic reflection of subsurface realities and the associated difficulty of delivering the remedial agent to where the contaminants of concern reside.

On the small-scale, the ISMA technology answers to this challenge by creating a platform for standardized flow-through sediment column experiments, and thus makes the more sophisticated continuous-flow evaluation method more accessible to the DoD and to the environmental restoration industry.

Technology Description. The ISMA is the hybrid of a laboratory treatability study and a field pilot trial. The device contains of all the components necessary for it to autonomously conduct a flow-through sediment column treatability study in the subsurface. All components – columns, pumps, electronics, etc. – have been miniaturized and assembled to fit within a 4-inch groundwater well. During operation, the ISMA is suspended in a well for approximately 4-8 weeks, during which time it operates autonomously collecting groundwater directly from the subsurface formation and feeding it into the array of microcosms. The ISMA can accommodate up to 10 sediment column microcosms, allowing for the side-by-side testing of 10 remediation strategies under truly identical conditions, or the testing of fewer strategies in replicate experiments to assess reproducibility. Throughout the deployment period, all the groundwater entering the ISMA is collected in column-specific, individual effluent capture vessels, which is analyzed in the laboratory after retrieval of the ISMA from the well.

The main advantages the ISMA offers are: (i) reduced cost when compared to alternatives; (ii) the opportunity to generate data on field performance of remediation technologies with zero-risk of negative impacts on the aquifer; (iii) the ability to screen multiple, mutually exclusive, treatment options in parallel; and (iv) to do so *in situ* using fresh groundwater, drawn in real-time from the subsurface formation, thereby reflecting the ambient hydrogeochemistry and microbiology of the target environment.

Limitations of the ISMA technology include that the current embodiment does not enable intermittent or continuous monitoring of conditions prevailing in the device during field incubation. Further, the construction of sediment microcosms may result in experimental bias and potential inactivation of sensitive anaerobic microorganisms. Lastly, as any other small-scale feasibility assessment tool, the ISMA technology is incapable of assessing site heterogeneities that are known to influence the outcome of remediation efforts.

Demonstration Results. Two demonstration deployment of the ISMA are summarized, one evaluating three different in situ remediation strategies for treatment of perchlorate, and the other evaluating three different strategies for treatment of two co-contaminants, hexavalent chromium [Cr(VI)] and trichloroethene (TCE). Where applicable, ISMA-generated results were compared to and found consistent with complimentary data sets produced from batch-bottle treatability studies, laboratory column studies, and field pilot trials.

Results gathered in the course of the project indicate that the ISMA is a cost-effective and suitable alternative to contemporary treatability or feasibility study methods. Qualitatively, results from ISMA and batch-bottle studies led to similar conclusions: both indicated that bioaugmentation was effective at treating the perchlorate (Site 1) and Cr(VI) and TCE (Site 2). This conclusion is consistent with the results from all relevant site-specific data sets, including (i) data gathered in our laboratory at Arizona State University from both complimentary batch-bottle studies and flow-through column studies; (ii) results generated from a batch-bottle study conducted by an outside consulting firm, (iii) and results generated from a field pilot trial. A quantitative comparison of first-order degradation rate constants found that batch bottles overestimated field rates by over an order of magnitude (>10), while the degradation rates observed in the ISMA differed from those observed in the field only by a factor of two (2). This result indicates that the ISMA more accurately reproduces field phenomena, and may potentially be used to quantitatively and accurately assess the field performance of *in situ* remediation technologies.

Cost Analysis. The report concludes with a cost-analysis of the ISMA demonstration deployments and a cost model for projecting future ISMA deployment costs. The cost-effectiveness evaluation finds that the ISMA costs are similar to a traditional bottle treatability study conducted in static (batch) mode, but notably lower than both a laboratory column test and field pilot trial. Furthermore, the standardized, modular components of the ISMA can be used as a platform for conducting column studies in the lab as well. This usage mode can serve to reduce costs of a laboratory column study, thereby making the more sophisticated flow-through evaluation method more accessible to environmental restoration professionals.

Summary. The ISMA is a new platform for conducting column studies in the laboratory and in the field. The standardized column format allows for the performance of experiments in multiple replicates, which is of great importance because of the large variability associated with microcosm experiments. The technology's high degree of automation reduces the requirement for constant monitoring by an operator. Its application in the subsurface helps to create quasi-field conditions in the device and eliminates to a large degree the need for maintaining expensive laboratory space; *in situ* operation may serve to reduce laboratory artifacts introduced by removal of groundwater from the subsurface. *In situ* operation also yields degradation rates that are more consistent with observed field rates, which will greatly benefit decision-making in the remedial design phase of site cleanup. Furthermore, the cost evaluation performed here showed that an ISMA deployment is only marginally more expensive than a contemporary batch bottle experiment but drastically less expensive than the alternatives, namely a contemporary laboratory column study and a field pilot trial.

1.0 INTRODUCTION

1.1 BACKGROUND

Swift and cost-effective remediation of contaminated aquifers is an important but challenging goal. It is widely acknowledged that *in situ* remediation strategies have to be tailored to individual sites based on their unique hydrogeological and biological conditions, as well as the types and concentrations of pollutants present (NRC 1993; ITRC 2002).

An initial screening of treatment approaches is typically accomplished with batch bottle microcosms, which feature a relatively simple design and low costs (ESTCP 2005). Batch microcosms offer determination of degradation rates with closed mass balances, and the number of sampling points and parameters is only limited by budgetary constraints. However, batch bottles cannot reflect flow-through conditions as they are encountered in the subsurface (U.S.EPA 1998). This can be accomplished in flow-through column microcosms that are filled with site sediment and amended with different treatment agents simulating *in situ* chemical treatment, biostimulation, or bioaugmentation. These types of studies are much more cost intensive than batch microcosm studies, and are therefore seldom used (Jackson, Garrett et al. 1984). If flow-through studies are conducted, often only one remediation approach is tested with no replicate studies.

All laboratory studies suffer from limited realism and results cannot simply be extrapolated to the field (Madsen 1991). Reasons for this limitation are numerous and include: removal of sediment and water samples from the aquifer can introduce chemical and biological changes; furthermore, heterogeneities at the field site are not addressed and the scale of laboratory tests is much smaller than the full-scale remediation later in the field. Therefore, results from laboratory studies need to be validated in field tests (NRC 2004).

A variety of approaches have been used for field testing of *in situ* remedies, including *in situ* microcosms (Gillham, Starr et al. 1990; Nielsen, Christensen et al. 1996), push-pull tests (Istok, Humphrey et al. 1997; Kleikemper, Schroth et al. 2002), and multi-well tracer tests (Ptak and Teutsch 1994; Amerson and Johnson 2003). Since field studies are conducted in an open system, mass balance calculations are limited by the quality of the monitoring network. Push-pull

experiments are a field study variation requiring only a single monitoring well; they have to be limited in time because of tracer recovery rates that decrease with both time lapsed and groundwater velocity (Schroth, Kleikemper et al. 2001). Due to the limited time an injected test volume of groundwater remains in the vicinity of the injection location, push-pull tests are not well suited for studying processes requiring long-term adaptation of microbial communities on the timescale of weeks and months (e.g., nutrient injection-induced anaerobic conditions in aerobic environments). Field tests vary in complexity and cost, but all suffer from an incapacity to produce truly comparable tests of competing remediation technologies. Different technologies can be tested in different wells / portions of the aquifer or sequentially at different times, but results are impacted by differing starting conditions or other unknowns (geochemical / microbial heterogeneity between different test wells, varying hydrology, contaminant distribution, etc.). Thus, there is a need for technologies that can compare different remediation strategies without impacting in any way the integrity of groundwater monitoring wells used for technology efficacy screening.

The tool we have developed is based on proven flow-through microcosm tests that are arranged in an array in the device (*in situ* microcosm array – ISMA), allowing multiple remedies to be tested side-by-side, thereby yielding scientifically comparable and statistically significant results. All components of a conventional laboratory column study were miniaturized and incorporated into a down-hole device. Everything entering the device is captured within the device, ensuring no impact on the groundwater well where the test is conducted. The ISMA can be deployed in any wells with a diameter of 4" ID or larger. A survey of groundwater wells in five states (Arizona, Texas, Minnesota, Pennsylvania and Illinois) showed that the majority of wells have dimensions of 4" diameter or larger. (Only wells up to 6" were considered in the survey, since larger wells are mostly used for water pumping purposes.)

The prototype version of the ISMA consisted of miniaturized capillary microcosms arranged in parallel and integrated into a self-contained apparatus that could be deployed in the field via suspension in a conventional groundwater monitoring well at a depth below the water table. The device consisted of a pump, an array of capillary microcosms arranged in 96-well format and an effluent bottle suitable for capturing the groundwater that passes through the device. Due to the

large number of capillary microcosms, multiple experiments could be conducted simultaneously in the target environment under *in situ* conditions. This prototype suffered from technical challenges (preferential water flow, cross-contamination between microcosms) and principal limitations (very short residence time, limited sample volumes), which were addressed in the current ISMA design described in this report, which was realized with ESTCP funding.

1.2 OBJECTIVE OF THE DEMONSTRATION

This demonstration is designed to validate the use of the *in situ* microcosm array (ISMA) technology for *in situ* screening of remediation strategies for contaminated aquifers. Field demonstration of the ISMA were performed with the objective to demonstrate that answering this novel technology can address key questions frequently posed by remediation regulators and decision makers:

- (i) Are contaminants being attenuated naturally, and if so, at what rate?
- (ii) Can this rate of contaminant removal be accelerated?
- (iii) Among the available active remediation approaches, which one will perform most favorably at the site?
- (iv) Will the manipulation of environmental conditions at the site lead to unwanted effects, such as sediment clogging or solubilization of toxic metals?

The principal objective of the technology demonstration was to show the feasibility and utility of comparing multiple remediation strategies *in situ* side-by-side in the same place at the same time. This document summarizes the design, execution and results of ISMA field demonstrations and may serve for potential end users as a general guide on how to utilize the ISMA technology in the design and interpretation of *in situ* feasibility studies.

1.3 REGULATORY DRIVERS

Regulatory drivers exist from the federal side as well as from state regulations. Sites regulated under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) have Substantive Requirements that are Operable Unit (OU) specific and that regulate the discharge of any water from that particular OU. This includes treated and untreated

groundwater as well as any reagents that have been added for treatment. The regulation also encompasses secondary groundwater contaminants whose concentrations could be affected by subsurface injection of substances for treatment. These secondary contaminants can include heavy metals, but also nitrate (NO_3^-), iron (predominantly in the water soluble form of Fe^{2+}) and other salts and metal ions. Regulations for non-CERCLA sites differ by state.

In Arizona, non-CERCLA site operators are required by the state to apply for an aquifer permit under Article 3 Aquifer Protection Permits Title 49-241, if they desire to inject any reagents into the subsurface. Under this rule, the injection of any reagents into the subsurface shall not lead to groundwater contaminants exceeding Aquifer Water Quality Standards described under Title 18-11-401. Arizona Underground Storage Tank (UST) regulations state the following:

“Information and conclusions drawn from pilot testing and feasibility studies should be provided, if appropriate, as justification for the selection of a remedial alternative. As used in this guidance, a feasibility study differs from a pilot study in that the certainty of success of the technology is more fully understood in the case of the latter. Pilot studies are typically conducted for a remediation technology to define the engineering parameters of system design and operation needed to accommodate site-specific conditions prior to full scale implementation.”

In California, regulations for non-CERCLA sites may vary between regions. For the San Diego region, Item 4 of the regulations states:

“The addition of materials to remediate ground water may require bench-scale and/or small-scale pilot testing prior to design and implementation of full-scale remediation. The addition of amendments to conduct pilot studies is covered under this Order.”

Under 'A. Conditions of Eligibility', states:

"Information on the possibility of any adverse impact to current or potential designated beneficial uses of groundwater quality, and whether the impacts will be localized and short-term [need to be determined prior to in situ treatment]".

For the California Central Valley, the California Regional Water Quality Control Board of the Central Valley Region regulates Pilot Studies by Order No. R5-2008-0149, "Waste Discharge Requirements General Order for In-Situ Groundwater Remediation at Sites with Volatile Organic Compounds, Nitrogen Compounds, Perchlorate, Pesticides, Semi-Volatile Compounds, Hexavalent Chromium and/or Petroleum Hydrocarbons".

One relevant regulation is stated under the *Conditions of Eligibility* and the other is in the *Notice of Intent Application*. In order to be eligible to conduct a full-scale enhanced bioremediation project, this order requires pilot studies of limited extent and duration to:

- 1) achieve the desired results and understand the reactant by-products or breakdown products; and
- 2) demonstrate that any by-products or breakdown products do not result in long-term adverse water quality effects.

The ISMA is designed as a state-of-the-art tool for conducting enhanced bioremediation treatability tests under realistic *in situ* conditions. The main objectives of this ISMA demonstration project were:

- 1) to demonstrate the feasibility of using the ISMA technology to simultaneously test multiple reagents and to determine an optimal reagent that will transform contaminants into benign by-products; and
- 2) to demonstrate the feasibility of assessing any unwanted water-quality impacts that could result from injecting a given selected reagent, and to do so without sacrificing a valuable monitoring location or irreversibly altering the water-bearing zone under investigation.

ISMA testing is designed to satisfy the pilot study eligibility requirements of state and governmental regulations by providing bench-scale and field testing and identifying possible adverse impacts to groundwater beneficial uses.

2.0 TECHNOLOGY

2.1 TECHNOLOGY DESCRIPTION

Treatability studies for *in situ* remediation are best accomplished in flow-through column microcosms that are filled with site sediment and amended with different treatment agents simulating *in situ* chemical treatment, biostimulation, or bioaugmentation. Their main advantage over conventional batch microcosms is the simulation of flow conditions, which govern processes in the subsurface. The ISMA technology is based on the proven column study approach (Drzyzga, El Mamouni et al. 2002) that is miniaturized here, such that fully controlled flow-through column experiments can be conducted in the field *in situ* (Halden 2004; Halden 2005). A column treatability study refers to a method of simulating field conditions in a controlled experiment whereby water continuously flows through a packed bed of sediment. The water and sediment can be collected from the actual location (well, subsurface stratum) being simulated, or one may use an analog or synthetic substitute prepared in the laboratory or collected elsewhere. Column studies represent the “gold standard” of laboratory treatability studies, owing to the continuous flow conditions they create that are more reflective of the subsurface.

The deployment of the *in situ* microcosm array (ISMA) technology encompasses:

- (i) the delivery of the self-contained ISMA device into the screened interval of a deployment well (Figure 2-1),
- (ii) incubation of the device for a period of several weeks,
- (iii) removal of the device from the deployment well, and
- (iv) analysis of the sediment columns contained therein, and of each columns’ effluent that is stored in the device in individual storage containers (effluent vessels) and retrieved from the well together with the ISMA apparatus after testing (Miller 2005).

A first prototype of the ISMA (further referred to as “Prototype ISMA”) was developed based on a 96-well format, which contained 96 capillary microcosms. The device was configured for high-

throughput analysis of protein and DNA to characterize the microbial community present in saturated subsurface environments.

The current ISMA device contains an array of up to 10 sediment columns configured to reflect different treatment approaches (e.g., natural attenuation, nutrient injection, bioaugmentation, passive reactive barrier, chemical oxidation, etc.) that may be mutually exclusive (Halden 2005). The ISMA further contains an intake with a one-way check valve, a 1-to-12 splitting manifold, 2 multi-channel peristaltic pumps regulating flow rates in 12 liquid lines, a step-motor delivering treatment agents, 12 separate liquid effluent capture vessels, 12 sorbent-based in-line cartridges for volatiles capture, secondary liquid containment system, and assorted control electronics and line management systems. The different components of the device are housed in tubular stainless-steel sections, which are connected sequentially during field deployment of the device (Figure 2-1). The connections between modules are load bearing, waterproof and transmit all necessary fluid lines and electrical signals. The device is suspended on a steel cable to the desired depth and electrical power is supplied from an array of batteries and solar panels in remote locations or from a standard electrical outlet (110 V or more) where available. This enables autonomous operation for the duration of the treatability test.

During the *in situ* test, groundwater is pumped directly from the subsurface formation through a screened intake (100 μm pore size), which is lined up in depth below ground surface (bgs) with the screened interval of the well or, in a well with a longer screen, with the depth where the treatment is to be implemented. Within the ISMA device, the groundwater flow is split into twelve individual lines by a custom manifold and fed through two six-channel peristaltic pumps, which pump the groundwater in an up-flow mode through the sediment-filled glass columns (microcosms). Up-flow operation ensures sediment saturation and allows gas bubbles to escape at the top of each column. Flow rates can be adjusted to achieve microcosm residence times representative of the linear velocity of groundwater in the targeted aquifer stratum at the deployment site.

Experiments in the ISMA are typically conducted in triplicate, producing data featuring confidence intervals that help to compare and identify in a scientifically defensible manner

which treatment works best. The device is designed such that conducted tests should leave no trace behind, do not change the local geochemistry and microbiology, and thus do not preclude technology-deployment wells from continued use as valid compliance monitoring locations (Halden 2005; Miller, Franklin et al. 2007). The ISMA represents the first tool that allows fully contained *in situ* flow-through studies with multiple approaches/replicates tested at the same time.

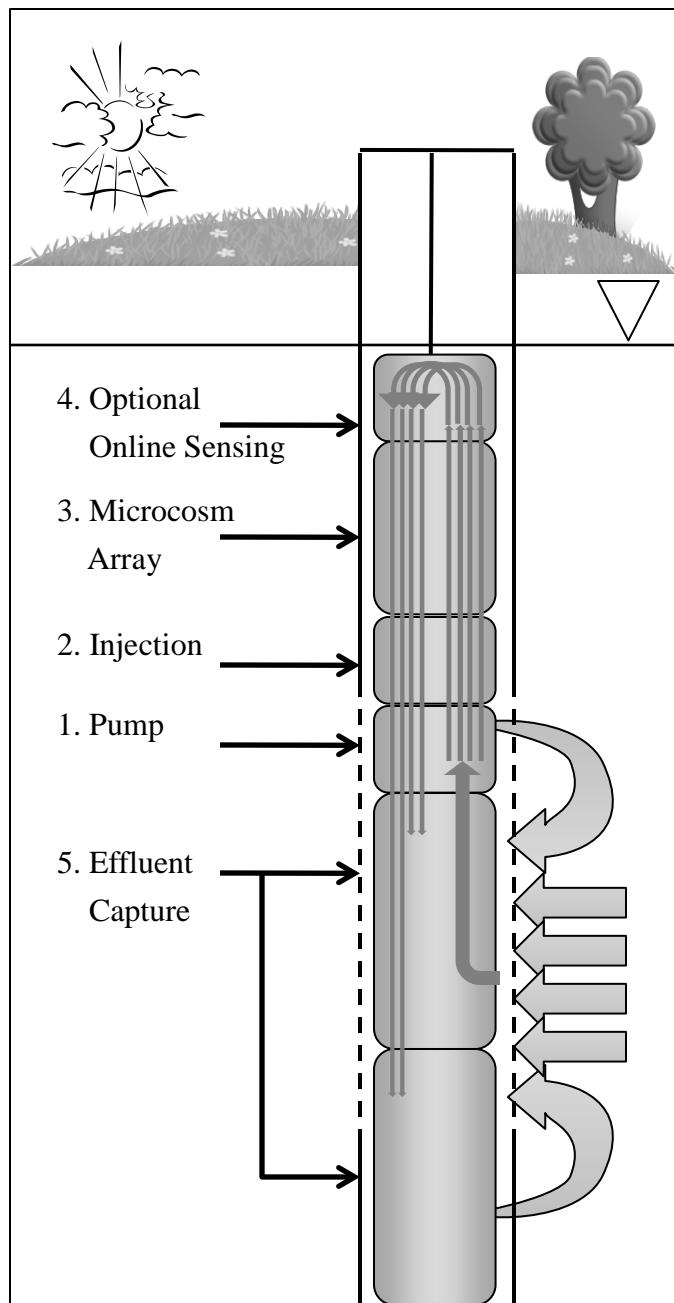


Figure 2-1. Schematic showing the *in situ* microcosm array (ISMA) apparatus suspended in a standard 4-inch (10 cm) inner diameter groundwater monitoring well. The device is supported from the surface via an umbilical tether, which holds it in place and provides power and control over the integrated multi-channel groundwater pumps. While deployed, desired test supplements (e.g., carbon sources promoting degradation of chloroethene), can be injected into sediment columns via the injection module, where they are allowed to interact with site groundwater and suspended microorganisms delivered by the peristaltic pumps. Upon retrieval, the contents of the apparatus can be analyzed (column effluent stored in effluent vessels; column sediment in microcosm array) to inform on processes that took place during *in situ* incubation.

The ISMA technology has been refined and tested in the laboratory for more than 8 years [2, 5]. Based on this research and development activity, a portfolio of intellectual property has been created with the goal of raising the attractiveness of commercial development of the technology so that it can be disseminate broadly to end-users by a commercial entity. The portfolio comprises one issued US patent (US7662618), 2 US patent applications (US20100159502, US20070161076), and 1 PCT application (WO2011097561). An overview of the technology has been provided through several platform presentations at premier national conferences (McClellan 2009; Kalinowski 2010; McClellan 2011; McClellan 2011; Kalinowski 2012; Kalinowski 2012; McClellan 2012; McClellan 2012), including a 4-hour workshop at the 8th International Conference for Remediation of Chlorinated and Recalcitrant Compounds on May 23rd 2012 (McClellan 2012). In addition, webinars have been conducted with the U.S. EPA (Aug 15th 2012) and for DuPont (Sep 19th 2012), with additional requests for webinar technology dissemination in development. These webinars can serve as technology primers and the full slide presentation including comments from potential end users are accessible online at: <http://www.clu-in.org/conf/tio/isma/>. Based on the positive feedback from the environmental restoration community, the ISMA technology has been funded by the Johns Hopkins University Technology Transfer Office, the National Institute of Health (NIH), the Maryland Technology Development Corporation (TEDCO) and the Environmental Security Technology Certification Program (ESTCP) of the Department of Defense (DoD), and the Biodesign Institute Commercial Translation program at Arizona State University. A startup company (ISW Technologies, LLC) has been formed to commercialize the ISMA technology and has negotiated exclusive rights to the ISMA patent portfolio. ISW Technologies, LLC is currently in discussions with major environmental remediation firms to develop strategic partnerships for ISMA placements at customer sites with anticipated rollout of the first customer deployments in the first half of 2013. The ISMA technology is designed to provide the DoD and other stakeholders in an inexpensive fashion with information that cannot be obtained in any other fashion. Information collected by the device on a well-by-well basis include:

- (i) occurrence and *in situ* rate of natural attenuation,
- (ii) identification among multiple (2 or more) treatment approaches that may be mutually exclusive (e.g., aerobic vs. anaerobic treatment), the one that is most effective in a given location,

- (iii) determination of the corresponding accelerated rate of contaminant removal,
- (iv) information on the extent of sorption and the migration of contaminants, injected nutrients and microorganisms in site sediment,
- (v) phenomena occurring as a result of treatment implementation (i.e., increased dissolution of toxic metals from site sediment), and
- (vi) information that is essential to conduct a cost analysis to understand which treatment is most economical, based on the gain in contaminant removal rate per volume of treatment agent.

2.2 TECHNOLOGY DEVELOPMENT

The first version of the *in situ* microcosm array (Prototype ISMA) was developed in our laboratory in 2004 (Halden 2004; Halden 2005). It consisted of miniaturized capillary microcosms that were arranged in parallel and integrated into a self-contained apparatus that could be deployed in the field via suspension in a conventional groundwater monitoring well at a depth below the water table. The device consisted of one pump, an array of capillary microcosms arranged in 96-well format and an effluent bottle suitable for capturing the groundwater that passes through the device (Figure 2-2). This Prototype ISMA had an integrated pressure-operated closure mechanism that allowed control of the flow of water in and out of the test compartments from the ground surface via an umbilical tether. Each Prototype ISMA sampler held 96 capillary microcosms, which could be operated in either batch mode, flow-through mode or a combination of the two (Halden 2004; Halden 2005). The umbilical tether connecting the ISMA to the surface allowed one to send a signal to the built-in closure mechanism and to the integrated water pump. Triggering of the device from the surface would cause the two valve plates to shift and the pump to start, thereby exposing each of the 96 capillary microcosms to a constant flow of groundwater (Figure 2-3). Microorganisms suspended in the water were forced into the capillaries filled with a filtration material (*e.g.*, machined, synthetic filter plugs). The filtration matrix could be coated with test substances diffusing from the inert matrix into the surrounding groundwater. Due to the large number of capillary microcosms, multiple experiments could be conducted simultaneously in the target environment under *in situ* conditions. The effluent of the various capillary microcosms was collected in a bladder contained in the effluent bottle at the bottom of the device (Figure 2-2). Owing to the presence of a

collection bladder, check valves and the unidirectional flow within the device, none of the effluent can escape into the surrounding groundwater.

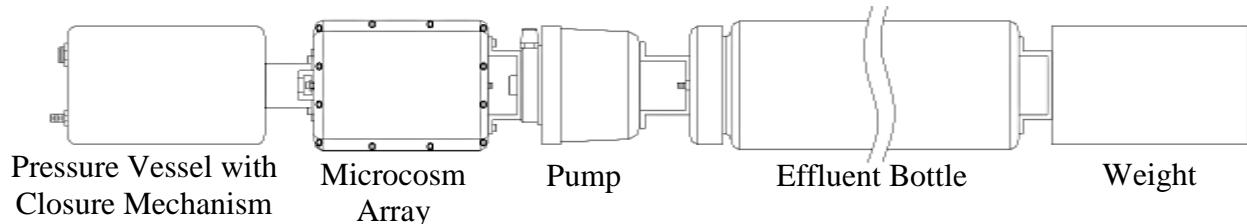


Figure 2-2. System components of an early embodiment of the *in situ* microcosm array (ISMA), showing a microcosm array that was based on a 96-well microtiter plate format.

Following retrieval of the tool from, e.g., a groundwater monitoring well, the device can be analyzed by chemical, genomic and proteomic techniques. Microorganisms were extracted for enumeration and characterization. Additional analysis on replicate capillary microcosms could be performed by MALDI-TOF MS analysis to determine the expression of pollutant-transforming catabolic enzymes (Halden, Colquhoun et al. 2005). Similarly, the effluent of each capillary microcosm and the content of the capillary itself could be analyzed chemically to determine biotransformation activity, but this was severely limited by the available sample volume.

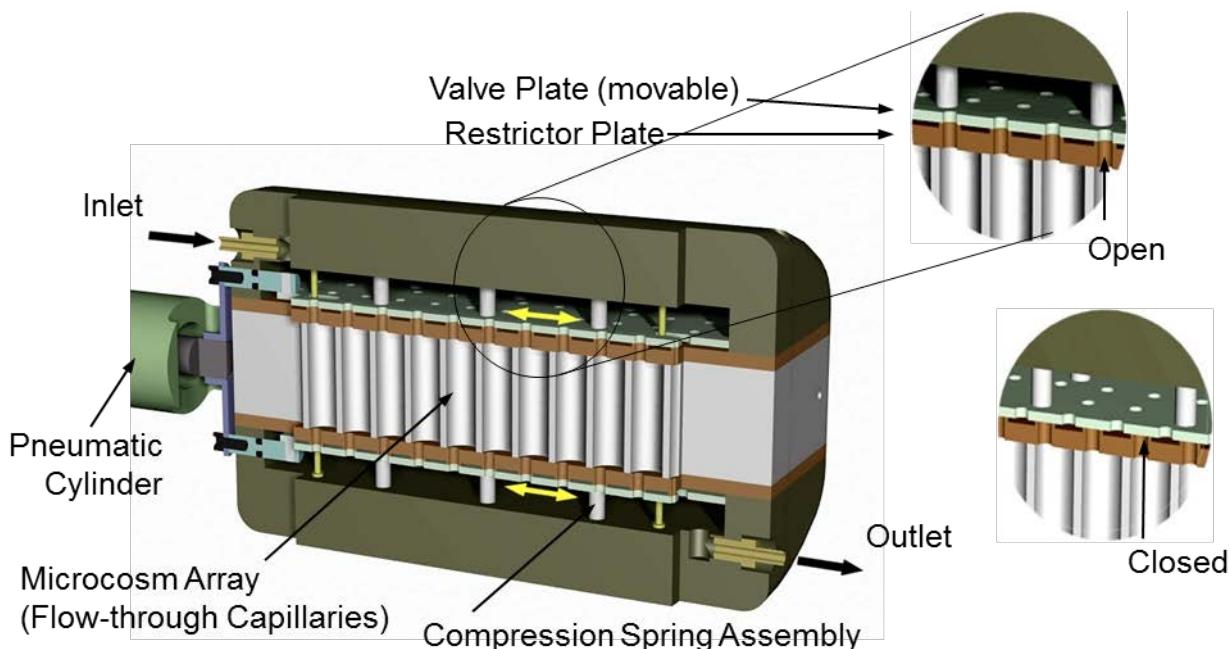


Figure 2-3. Cutaway view of the closure mechanism and the microcosm array of the Prototype ISMA (see text for details).

The Prototype ISMA served the purpose of conducting initial proof-of-principle studies. In this work, a number of limitations of the Prototype ISMA were identified and later overcome during unfolding of this project that resulted in development and testing of the current ISMA hardware. These generational hardware improvements included:

- elimination of potential for cross-contamination between microcosms by providing separate tubing and pump channels for each microcosm, which stay separated throughout the device
- elimination of possibility of preferential flow through any one of the 12 channels through the use of multi-channel peristaltic pumps which deliver flow to each channel independently
- increased residence time through the use of larger microcosms (1 x 25 cm glass columns) compared to 96-well format (6.5 x 10.67 mm wells)
- simpler and safer containment of sediment in the microcosms; sediment is packed into each microcosm and kept in the glass column by a 100- μ m filter and Teflon® plug
- larger diameter (1 cm instead of 6.5 mm) to minimize wall effects in the column
- larger sample volume collected allowing for a range of chemical analyses (up to 1-L sample volume per microcosm)

2.3 ADVANTAGES, RISKS AND LIMITATIONS OF THE TECHNOLOGY

The ISMA detailed in this report represents the first embodiment of the device that was successfully applied in the field. The technology offers several attractive attributes that are unmatched by other available, contemporary technology screening approaches. Yet, like any technology, the ISMA also has specific risks and limitations that are discussed in the following.

Context and Problem Statement. No hazardous waste site is like another. Distinguishing features of individual sites include a unique climate, geology, hydrology, microbiology, contaminant profile and release history as well as the implications and remnants of any previous treatments implemented (NRC 1997). This situation challenges the DoD to produce customized solutions for one-of-a-kind problems. Since laboratory treatability studies are expensive and provide mostly qualitative rather than quantitative data, they often have to be followed by field tests (Vancheeswaran, Yu et al. 2003). Field-testing of a single technology assumes that the best solution already is known, when indeed this may not be the case. Conventional treatability

studies for *in situ* remediation projects usually involve laboratory batch microcosm experiments and/or limited-scale field studies. Field studies may consist of "push-pull tests" where a reagent is injected into the subsurface and then extracted from the same well after residing in the subsurface for some period of time.

Advantages. The value of the ISMA, as demonstrated here, lies in its ability to test multiple reagents simultaneously under representative subsurface conditions without irreversibly impacting the contaminated water-bearing zone in the vicinity of the well and without sacrificing the well as a monitoring point. The ISMA technology provides multiple benefits (Table 2-1). It represents an alternative to the aforementioned 2-step process (laboratory studies followed by field tests) by allowing parallel testing of multiple treatment approaches in the field. This combines the advantages of proven laboratory treatment test systems with the realistic physical, chemical, and biological conditions found in the field. This approach may be associated with significant cost savings for laboratory personnel and infrastructure, as the tests are conducted autonomously in the subsurface. In contrast to conventional field tests, where multiple treatment technologies are evaluated in different locations (i.e., different monitoring wells) to prevent the various tests from interfering with each other, the ISMA was designed to allow for testing of different treatments in the same well at the same time using the same groundwater as influent.

This design thus should enable the decision maker to make a fair comparison of treatment strategies, as well as to assess contaminant degradation under realistic conditions, i.e., the specific conditions prevailing *in situ* in the subsurface. Another claimed advantage of the device and technology is that the well used for testing is not put at risk of becoming compromised from treatment agent injection, sediment clogging or other potential problems that frequently are associated with conventional field tests. After ISMA tests have been performed, the deployment location is believed to be unchanged and thus may resume its original function of, for example, serving as a compliance monitoring location, as demonstrated here.

Significant variability may be observed in the outcome of conventionally conducted treatability tests performed in multiple columns in replicate. Therefore, results from some tests may be difficult to interpret or inconclusive. In microcosm experiments, large differences in the outcome

of identical experiments are common. The ISMA technology is designed to quantify this variability in outcomes by conducting experiments in multiple replicates (typically three) and by doing so *in situ*, to capture phenomena that cannot be easily reproduced in the laboratory environment.

Another common problem in subsurface bioremediation is sediment clogging due to excessive microbial growth, a situation often triggered by the injection of nutrients during biostimulation and bioaugmentation. It was proposed as a distinct advantage of the ISMA that one can study and diagnose clogging phenomena with this device by analyzing hydraulic sediment parameters pre- and post-deployment.

To approximate natural conditions, ISMA microcosms preferably are constructed from sediment obtained from the site and the specific subsurface stratum of interest. A number of processing options exist for the sediment material chosen for flow-through column microcosms, including the use of air-dried sediment and the use of only a specific grain-size fraction of the sieved and air-dried site sediment. Alternatively, sediment cores also can be obtained by pushing the ISMA glass columns into a sediment core obtained from a recently drilled undisturbed borehole core; while conceptually possible, this specific application was not part of this demonstration project. Each sediment-processing procedure offers its distinct advantages and disadvantages. Therefore, it may be desirable, and certainly is technically feasible, to conduct *in situ* column studies with flow-through microcosms constructed from differently treated site sediment materials.

To minimize the occurrence of laboratory artifacts such as oxygen intrusion, loss of microbial biomass from predatory grazing during groundwater storage, etc., the ISMA is deployed *in situ*. This strategy allows for direct entry of site groundwater into the down-hole device in real time without the need for groundwater transfer to the ground surface or to the laboratory.

Still, the construction of sediment microcosms may result in experimental bias and potential inactivation of sensitive anaerobic microorganisms. The problem of microbial inactivation is of particular concern when dealing with strict anaerobic microbes such as microorganisms of the group of *Dehalococcoides*. There also is a risk that, due to slow growth and kinetics, the *in situ*

incubation period may be too short to observe detectable changes in contaminant concentrations in the ISMA. This problem may be addressed by increasing the incubation time and by adding microorganisms to accelerate biotransformation kinetics via increases in biomass. We have also explored the option to inoculate and incubate sediment columns in the laboratory prior to field deployment to reduce uncertainty of a sufficient *in situ* incubation period. However, this approach negates some of the cost benefits described earlier. This option is further described in sections detailing the technology demonstration at Naval Air Station North Island.

Risks. The potential safety risks associated with the ISMA technologies are few and only minor. Risks may be posed by unwanted chemical release of chemicals during ISMA deployment in the event of leakage from the device. The amount of chemical agents and biomass in the sediment microcosms of the ISMA apparatus is very small and does not pose much risk. The preservatives/quencher used in the effluent collection bladders for stopping of biological and chemical reactions potentially could pose a minor risk, if released into the well. However, a non-human-toxic preservative (Kathon®) can be used to minimize those risks). Additionally, leakage of internal components would cause spillage of chemicals into the ISMA housing only. The dual containment design virtually eliminates the risk of chemical release into the subsurface environment.

Limitations. One perceived limitation of the ISMA technology is that the current embodiment does not enable intermittent or continuous monitoring of conditions prevailing in the device during field incubation. Instead, chemical and biological signatures are being collected over time and are analyzed after retrieval of the device to yield composite samples and thus composite data. The lack of feedback from the device during operation can make it difficult for the operator to know when to retrieve the device, i.e., at what time a given reaction has progressed sufficiently or has come to completion. This limitation potentially may be addressed in several ways. One solution would be for the operator to periodically retrieve the device to obtain updates on the extent of chemical and biological reactions taking place in the device. Another option would be develop a real-time monitoring module that queries the chemistry in the various column effluent lines sequentially. Alternatively, in shallow deployment situations, effluent from

the columns may be transported to the surface for monitoring. New hardware and software solutions for the ISMA technology are being developed at Arizona State University.

The second limitation of the ISMA is common to any and all small-scale feasibility studies, whether conducted in the laboratory or in the field. The ISMA technology is incapable of assessing site heterogeneities that are known to influence the outcome of remediation efforts. It does not address the hydraulic communication between wells within the footprint of the plume under remediation. Often times, preferential pathways in the form of permeable sand lenses or bedrock fractures, can profoundly impact the overall performance of in situ remediation projects. Unfortunately, these features are not easily detected using conventional site characterization methods. Injecting a remediation reagent into the subsurface at a poorly characterized site will not only make it difficult to assess performance, it also could displace the plume in unpredictable ways. Spatial heterogeneity can be approximated using tracer experiments and/or through a series of hydraulic stress tests. In order to maximize the chances for success of an in situ remediation project, it is crucial to develop a defensible hydrogeologic conceptual model of the subsurface that accurately reflects the hydraulic communication between wells within the project before implementing a given remediation approach at the field-scale. Yet, what is being learned at one well cluster may not be representative of the remainder of the site under investigation. Thus, there are some aspects of in situ remediation that can only be fully understood and appreciated during full-scale implementation of the selected treatment strategy. The ISMA can serve to identify the most promising treatment approach and to approximate the rate and extent of remediation that may be achieved. During full-scale remediation, additional information will be obtained that in some instances may require adjustments and modifications of the selected remedy in order to meet the desired treatment goal. The reader may refer to Table 2.1 for additional information.

Table 2-1. Overview of principal advantages and limitations of ISMA technology.

Advantages	Addressable Limitations	Inherent Limitations
<ul style="list-style-type: none">• Does not impact deployment well• Tests multiple treatments simultaneously in same well• Uses fresh groundwater not altered through handling/storage or transport to the surface• Generates all test data at the exact temperature prevailing at the site• Can use real site sediment• Can approximate potential for sediment clogging• Very low risk for site owner & field personnel• Parallel replicate experiments yield Statistically significant results• Provides field testing data at a cost comparable to experiments conducted in the laboratory	<ul style="list-style-type: none">• Lack of real-time data• Gathering of discrete samples currently is limited and would require temporal removal of the tool from the target depth• Limited residence time / <i>in situ</i> incubation, dictated by sediment column length, flow rate selected and sediment porosity• A complete mass balance for volatile compounds is hindered due to losses from off-gassing	<ul style="list-style-type: none">• Sediment characteristics may be altered by transfer to the surface and processing/storage in the field or laboratory• Representation of subsurface heterogeneities is limited to the cm-range (column length of 25 cm unless used in series) and requires use of undisturbed sediment cores that were not evaluated in the present study• Cannot be used to determine radius of influence (ROI) or the capacity of a formation to receive amendments.

3.0 PERFORMANCE OBJECTIVES

The following section lists the performance objectives set for the ISMA technology demonstration. A summary of the results is given in this section. For a detailed discussion of the performance assessment, please refer to section 6.

Table 3-1. Performance Objectives as stated in the Demonstration Plan.

Performance Objective	Data Requirements	Success Criteria	Results
Qualitative			
Demonstrate capability of conducting mutually exclusive experiments in parallel in the same well	Monitoring of select water chemistry parameters	Evidence for mutually exclusive conditions in parallel experimental groups (i.e. aerobic, anaerobic).	Objective met
No residue released into monitoring well during testing	Water sampling and chemical analysis before and after ISMA deployment	Groundwater chemistry does not differ before and after ISMA deployment	Objective met
Determine potential side effects of remediation strategies	Monitoring data for potential adverse outcomes (e.g., heavy metal dissolution and leaching)	Mass balance for secondary contaminant (e.g., VC accumulation, Cr leaching) in various experiments reveal quantitative data for different simulated remediation approaches	Objective met
Quantitative			
ISMA study is cost-effective compared to a lab study of comparable scope	Compile cost data for ISMA and lab study	Cost of ISMA study is equal to or less than cost of lab study of comparable scope	Objective met
ISMA study is cost-effective compared to a field trial producing a similar data output	Compile cost data for ISMA and field trials	Cost of ISMA experiment is equal to or less than cost of field trial	Objective met
Reproduce outcome of prior lab studies in the ISMA	Monitoring of select water chemistry parameters	Available rates and trends determined in the lab can be reconciled with ISMA results. Rates between field and ISMA agree within an order of magnitude.	Objective met
Reproduce outcome of prior field trials in the ISMA	Monitoring of select water chemistry parameters	Available rates and trends determined in the field can be reconciled with ISMA results. Rates between field and ISMA agree within an order of magnitude.	Objective met

3.1 PERFORMANCE OBJECTIVE: Demonstrate capability of conducting mutually exclusive experiments in the same well

The treatability tests inside the ISMA are all performed in parallel, i.e., the same water is fed as influent to all microcosms at the same time in the same well at the same temperature. The ability to conduct such sophisticated treatability trials in parallel is a unique capability, and represents an important advance over established methods for treatability testing (Halden 2004; Halden 2005; Halden 2005).

3.1.1 Data Requirements

To evaluate the success of this objective, data are needed on the remediation effectiveness of each individual treatment approach conducted in parallel.

3.1.2 Success Criteria

In this report, success of the ISMA technology was defined as a demonstration that the device is indeed capable of evaluating the effectiveness of multiple remediation strategies by conducting trials of at least two different strategies in parallel.

3.2 PERFORMANCE OBJECTIVE: No residue released into monitoring well during testing

A major advantage of the ISMA technology is the ability to conduct *in situ* treatability testing without changing the groundwater chemistry in the well. The ISMA is designed to be completely self-contained and therefore is expected to have no impact on potential future use of the deployment well for monitoring purposes.

3.2.1 Data Requirements

Lack of release of any residues into the monitoring well during ISMA deployment was demonstrated by analyzing groundwater collected from the demonstration site pre- and post-ISMA deployment.

3.2.2 Success Criteria

An observed lack of unexpected changes in groundwater chemistry pre- and post- technology deployment was postulated to constitute success.

3.3 PERFORMANCE OBJECTIVE: Determine potential side effects of remediation strategies

It is known that the amendment of subsurface sediments with chemicals and/or biomass potentially can have unintended effects on groundwater chemistry. For example, secondary contaminants can arise from incomplete degradation of the primary (initially present) contaminant to a degradation product that still presents a hazard. Secondary contaminants can also be metal salts that are present in solid form but become dissolved due to changes in redox conditions or pH as a result of implemented treatment approaches. This can result in elevated levels of alkalinity, hardness, or heavy metals such as chromium and arsenic.

3.3.1 Data Requirements

To assess any given side effects of different remediation strategies, concentrations of heavy metals and toxic degradation products need to be monitored in treated groundwater samples. For the candidate demonstration sites, known potential secondary contaminants are hexavalent chromium [Cr(VI)] and vinyl chloride.

3.3.2 Success Criteria

Demonstration of the capability to track the accumulation of secondary contaminants and calculate contaminant fluxes through differently amended sediment microcosms was defined as a measure indicating successful demonstration.

3.4 PERFORMANCE OBJECTIVES: ISMA study is cost-effective compared to a lab study of comparable scope

The relative cost to generate usable data is a key factor in determining the value of the ISMA as a diagnostic remedial design technology. Consequently, we performed a cost assessment for the ISMA and determined the cost to generate a comparable dataset by conducting a lab study.

3.4.1 Data Requirements

For this project, it was necessary to collect data pertaining to the cost to carry out each set of experiments, including the cost of personnel, laboratory space, analytical equipment, and consumable materials.

3.4.2 Success Criteria

With respect to cost considerations, success was defined as an outcome in which the cost to generate an equivalent dataset with a lab trial similar to one produced by an ISMA deployment are either equal or higher.

3.5 PERFORMANCE OBJECTIVES: Compare cost of conducting ISMA study vs. field trial

The relative cost to generate usable field data is a key factor in determining the status of the ISMA as a viable technology. Consequently, we performed a cost assessment for the ISMA and for the respective field trials that are the basis of our qualitative performance objectives.

3.5.1 Data Requirements

For this project, it was necessary to collect data enumerating the cost to carry out each set of experiments, including the cost of personnel, laboratory space, analytical equipment, heavy machinery and consumable materials.

3.5.2 Success Criteria

The ISMA has to be considered a viable technology if demonstration data show cost parity or cost savings with a field trial that would generate a comparable dataset.

3.6 PERFORMANCE OBJECTIVE: Reproduce outcome of prior lab studies in the ISMA

3.6.1 Data requirements

In order to assess the outcome of the ISMA experiments it was necessary to replicate prior lab studies, similar parameters as in the bench scale tests were monitored, such as concentration of chlorinated solvents, hexavalent chromium, and other suitable parameters.

3.6.2 Success criteria

The experiments were considered a success, if the rates generated in the lab and ISMA trials agree within an order of magnitude.

3.7 PERFORMANCE OBJECTIVE: Reproduce outcome of prior field trials in the ISMA

3.7.1 Data requirements

In order to assess the outcome of the ISMA experiments it was necessary to replicate prior lab studies, similar parameters as in the bench scale tests were monitored, such as concentration of chlorinated solvents, hexavalent chromium, and other suitable parameters.

3.7.2 Success criteria

The experiments will be deemed successful if the rates generated in the field and ISMA trials agree within an order of magnitude.

4.0 SITE DESCRIPTION

4.1 Site Selection

For the ISMA demonstration, we selected two sites, one in California and one in Arizona. Information on selection criteria and requirements can be found in the Site Selection Memorandum provided in Appendix C.

4.2 Site Location and History

4.2.1 NAS North Island, San Diego CA – OU-20

NAS North Island (NAS-NI) is located in San Diego County, California, southwest of the city of San Diego, on the tip of the Silver Strand peninsula with the city of Coronado adjacent and to the east. The remainder of NAS North Island is surrounded by water, the Pacific Ocean to the south and San Diego Bay on the west and north (Figure 4-1). North Island was commissioned in 1917 and is currently an active military base. Since 1935, NAS North Island has been occupied exclusively by the Navy. Operable Unit 20 (OU-20) is located in the northeast portion of the island.

Industrial processes performed in Buildings 1 and 2 at OU-20 are the likely source of hexavalent chromium in groundwater. Past operations at Building 1 were related to helicopter blade repair and maintenance, as well as the manufacture and repair of fiberglass components. Activities included parts grinding, cleaning, anodizing, paint stripping, and painting. Liquid wastes and rinse waters from these operations were piped to the Industrial Waste Treatment Plant via an industrial waste pipeline that was discovered to have breaks in it (BNI, 2005a). Additional contributions to the subsurface contamination may have included overflow of subsurface pits used for temporary waste storage, and outdoor aircraft fuel tanks washing



Figure 4-1. Regional Location Map of NAS-NI.

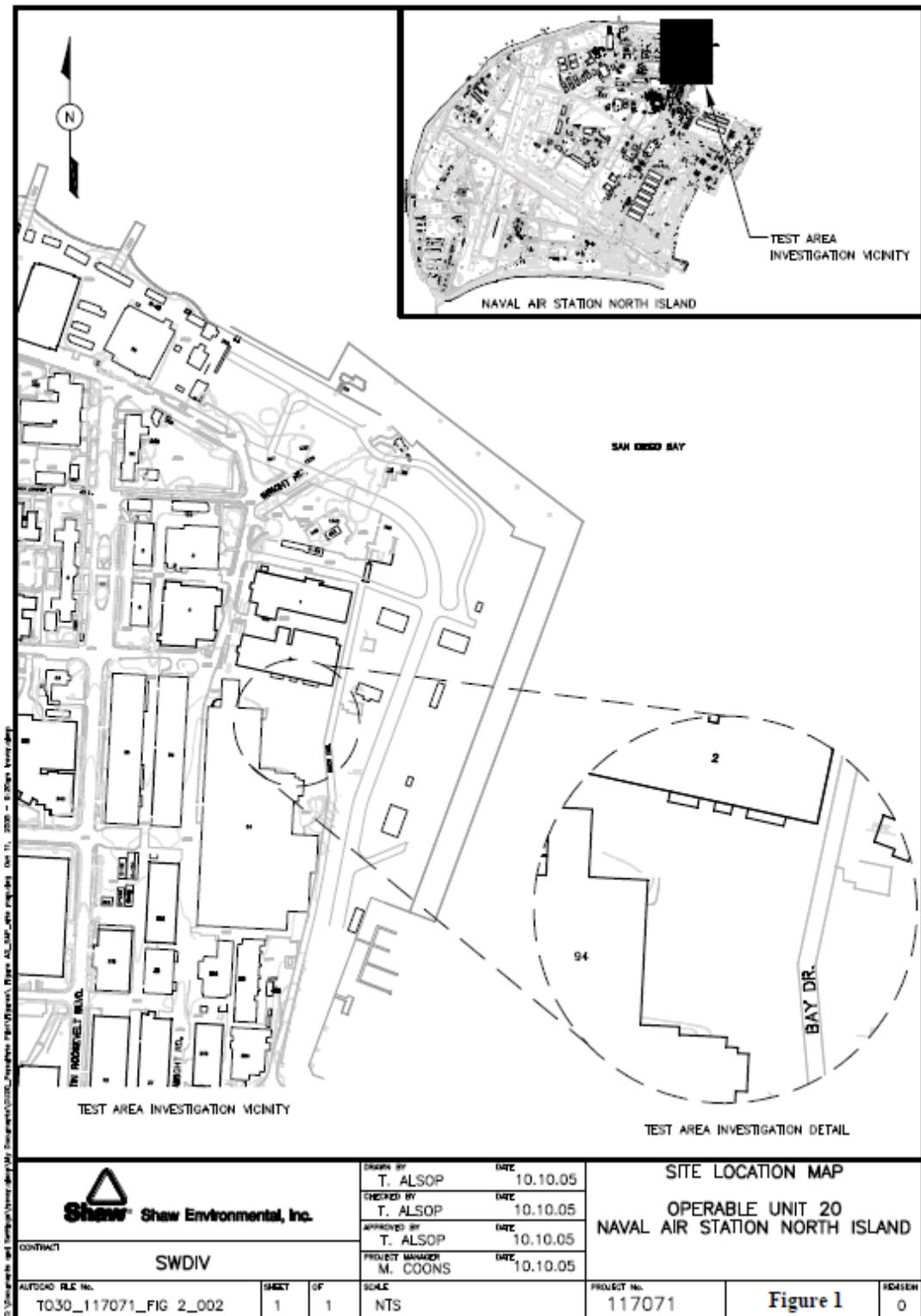


Figure 4-2. Site location map – OU 20.

4.2.2 Industrial Site, Mesa, AZ

This site is located outside the City of Mesa in Maricopa County, AZ and is home to an aerospace company, which designs, develops, and manufactures aircraft escape rocket motors and rocket catapults for emergency escape and survival systems, including the required propellants, among other products. The company has been located at the site since 1960.

Historically, water and solids generated by the processes on site were discharged to two unlined sludge beds, designated as water bore-out (WBO) pits, located approximately one-quarter mile east-northeast of the main Plant #3 facilities. The area of the two pits was approximately 60 ft long and 55 ft wide. WBO operations were conducted in a concrete building located approximately 200 feet northwest of the pits. A suspension of water and solid rocket fuel generated during WBO operations at the building were discharged to the pits. In the late 1990s, surface soil removal and confirmation soil sampling was conducted to facilitate site closure by the Arizona Department of Environmental Quality, followed by backfilling and leveling the area to surrounding grade.



Figure 4-3. Site Map – Industrial Site.

4.3 Site Geology and Hydrogeology

4.3.1 NAS North Island – OU-20

NAS-NI is located on relatively flat land with an average elevation of approximately 20 feet above sea level. The island was enlarged beginning in the 1930s through placement of hydraulic fill dredged from San Diego Bay onto tidal flats and nearshore areas. All of NAS-NI has been graded for development, and the area surrounding Buildings 1 and 2 is covered with asphalt, concrete, or maintained landscaping. The hydraulic fill used to construct much of NAS-NI consists of medium-grained to coarse-grained, poorly graded sands and silty sands. In some areas, the fill is underlain by organic silts and clays.

Since most of NAS-NI is paved, groundwater recharge is minimal and occurs primarily from irrigation. Shallow groundwater beneath NAS-NI is unconfined, and groundwater occurs at depths from approximately 4 to 25 feet below ground surface (bgs). Groundwater in the investigation area flows northeast and discharges into San Diego, not accounting for temporary fluctuations due to tidal influence.

The groundwater level in OU-20 is approximately 5 feet above msl. The groundwater gradient across the study area is relatively flat and ranges from 0.001 to 0.002 foot per foot. Groundwater flow direction is to the north/northeast. Aquifer transmissivity values calculated from slug and pumping tests in the Building 379 area ranged from 0.5 to 1,116 square feet per minute (ft^2/min), with an approximate value of $418.5 \text{ ft}^2/\text{min}$ calculated nearest to the ISMA deployment location (well S1-MW-9) (SES-TECH 2010).

4.3.2 Industrial Site, Mesa, AZ

Industrial facilities are located within the Basin and Range Physiographic Province, which is dominated by a series of northwest-trending mountain ranges and alluvial valleys containing thousands of feet of unconsolidated sediments (Consultants 1988). The Province was formed during Middle Tertiary time and evolved as a result of complex structural movements and associated erosion and deposition events (Society 1987).

Groundwater from the regional alluvial deposits is used for irrigation, as well as for industrial and municipal supply purposes. Two water wells and three monitor wells exist within approximately one-half mile of the site. The Salt River Project (Project 1990) interpreted the direction of regional groundwater flow to be in a southeastern direction, towards a groundwater pumping station located south of Falcon field airport. The depth to groundwater in the regional alluvium is approximately 225 to 275 feet below ground surface (bgs).

The site is situated on the eastern edge of the East Salt River Valley Groundwater Basin. The regional hydrostratigraphy consists of the Upper Alluvial Unit (UAU), the fine-grained Middle Alluvial Unit (MAU), and the Lower (Conglomerate) Alluvial Unit (LAU). The MAU is reportedly not present in the vicinity of the former WBO Pits (Basin & Range Hydrogeologists, 1991). The UAU ranges in thickness from about 265 to 685 feet in the vicinity of the site, and the LAU, which overlies granitic basement rocks, ranges from about 100 to 125 feet thick. The geology of impacted zone (UAU) is made up of unconsolidated to moderately well-consolidated sand and gravel, with variable amounts of finer material or larger cobbles and boulders.

Groundwater at the site is present under unconfined conditions at depths of about 175 feet below ground surface (bgs), based on wells installed in early 2009 (WBO-1, HPA-1 and NT-1; see Figure 4-4). Groundwater elevation trends in the vicinity of the site indicate increases on the order of 7 to 8 feet per year in recent years (Caldwell 2009). This trend is also observed on a more regional scale, based on groundwater elevation records maintained by the Arizona Department of Water Resources. The rising water table is attributed in part due to the Granite Reef Underground Storage Project, that is located only about 2 miles northwest of Plant #3. Groundwater flow is to the south-southeast, based on localized water level data from the above mentioned wells, larger scale water level surveys (Terranext 2007) and regional flow modeling. The hydraulic conductivity of the upper-most portion of the subject aquifer is estimated to be about 25 ft/day, based on the hydraulic testing conducted at well WBO-1 in February 2009 (Consultants 2009).

4.4 Contaminant Distribution

4.4.1 NAS North Island – OU 20

The OU-20 VOC and Cr(VI) plumes is located in the northeastern portion of NAS North Island. The VOC plume originates from the vicinity of Building 379 and extends downgradient to the northeast approximately one half-mile, with several sources contributing. The Cr(VI) plume originates in the vicinity of building 2, with the former anodizing shop in Building 2 as the most likely source of Cr(VI), and extends downgradient approximately 700 ft (Figure 4-4).

The ISMA deployment well OU20-PEW-01 is located on the southwest edge of the chromium plume, in the parking lot located between buildings 2 and 94, and marked in Figure 4-4 with a red circle. This well was chosen because it was (i) preexisting, (ii) sufficiently sized to accommodate the ISMA, (iii) outside and up-gradient of the field pilot-test areas, and (iv) minimally disruptive to traffic and logically easy to access due to its location in a parking lot.

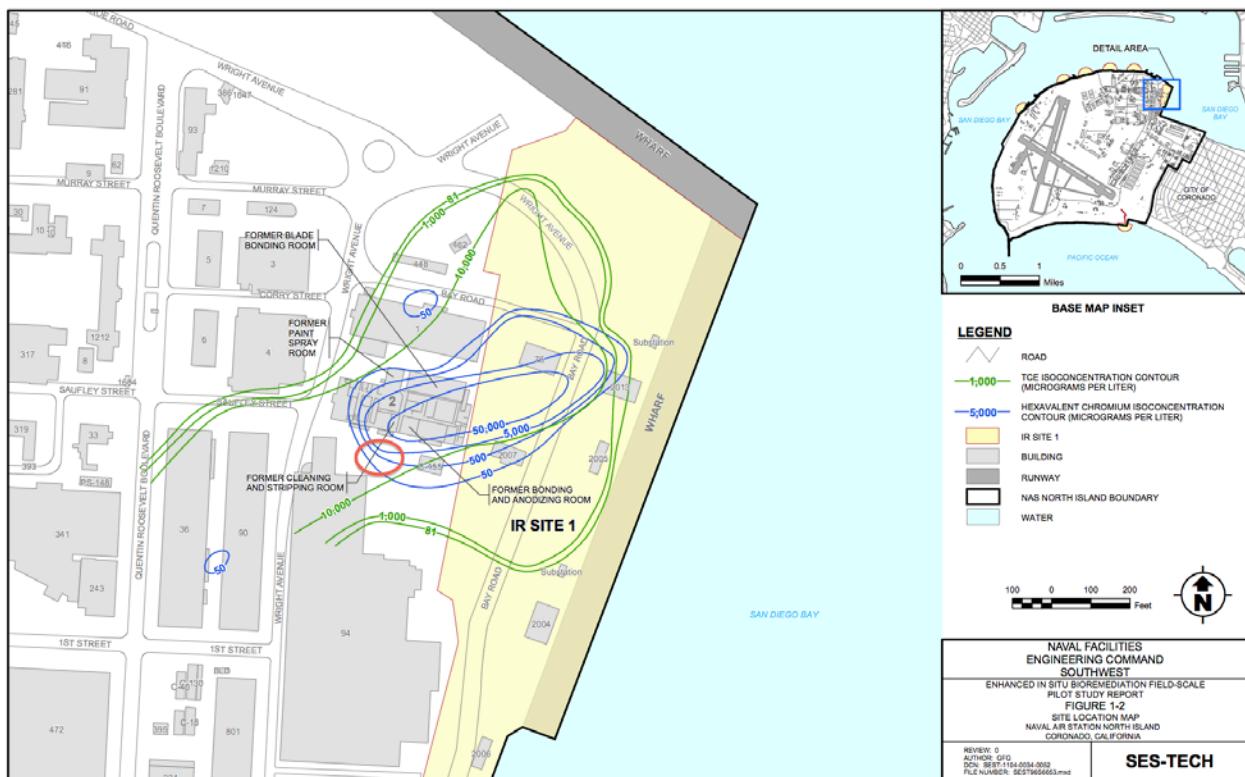


Figure 4-4. OU 20 TCE and Cr(VI) Plumes at OU-20.

4.4.2 Industrial Site, Mesa, AZ

During the installation of monitoring well WBO-1 in 2009 (Figure 4-4), soil samples were collected for detailed delineation of key constituents in vadose zone soils, including perchlorate, ammonium, nitrate and pH (Consultants 2009). The results can be summarized as follows:

Perchlorate: Concentrations in the WBO-1 soil samples ranged up to 1,525 mg/kg. Peak concentrations were detected within a depth interval of 60 to 90 feet bgs, with concentrations exceeding 200 mg/kg extending from 90 feet bgs to 170 feet bgs, just above the water table.

Ammonium: Elevated concentrations of ammonium (up to 2,220 mg/kg - as nitrogen) were detected in soil samples collected from a depth interval of 40 to 60 feet bgs. The transport of ammonium has however been retarded relative to the transport of perchlorate, as evidenced by the different depths of the peak concentrations of these constituents.

Nitrate and Nitrite: Elevated concentrations of nitrate (up to 360 mg/kg – as nitrogen) were detected at depths of 40 to 60 feet bgs, which likely corresponds with the elevated ammonium levels. Below 100 feet bgs, all reported nitrate concentrations were less than 15 mg/kg. Nitrite was not detected in any of the soil samples.

pH: The pH of the WBO-1 soil samples ranged from 5.7 to 9.1, with pH generally increasing below the zone of elevated ammonium. It should be noted that a decrease in pH is anticipated during nitrification of ammonia/ammonium.

Perchlorate concentrations found during multiple sampling campaigns in monitoring wells in the plume area are listed in Table 4-1. Combined with the current rise in groundwater elevation (about 8 feet annually), it appears that perchlorate groundwater impacts in the WBO area are primarily a result of the rising water table saturating overlying perchlorate-impacted soils, rather than from perchlorate percolating downwards through the overlying vadose zone soils.

Table 4-1. Monitoring well data at Site 2.

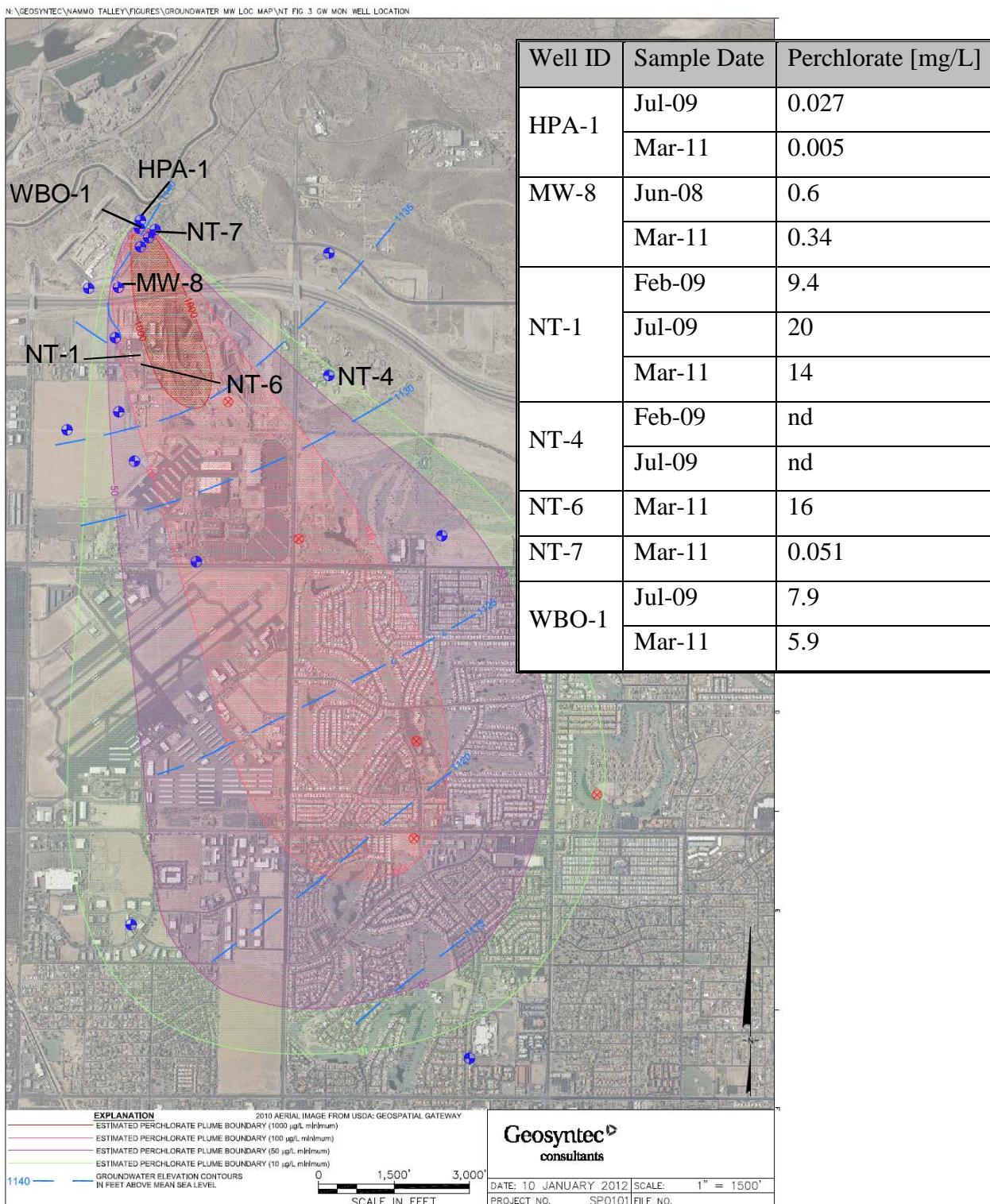


Figure 4-5. Perchlorate plume at Site 2.

5.0 TEST DESIGN

5.1 CONCEPTUAL EXPERIMENTAL DESIGN

As detailed in Section 3, the primary goals of the described ISMA demonstrations were related to showcasing the functionality of the ISMA, and the secondary goals were to compare the data output of the ISMA to the extant data sets associated with the two deployment sites. Accordingly, the treatability experiments conducted in the ISMA were designed to be as comparable as possible to the extant lab and field treatability data sets associated with the deployment location.

The deployment of the ISMA technology encompasses: (i) the delivery of the self-contained ISMA device into the screened interval of a deployment well (Figure 1); (ii) incubation of the device for a period of several days to weeks; (iii) removal of the device from the deployment well; and (iv) analysis of the miniature sediment columns contained therein, and of each column's effluent that is also stored in the device and retrieved from the well together with the ISMA apparatus after testing (Miller 2005). The current version of the ISMA hardware used in these demonstrations has 12 liquid flow channels. Up to 10 of those channels can feed sediment columns, with the remainder of the channels allocated for collection of untreated groundwater to serve as a baseline or control. Furthermore, up to 6 of the liquid lines can be continuously amended with an *in situ* agent throughout the deployment period.

The 12 lines can be allocated between experimental groups as necessary to optimize between the number of experimental groups (i.e., number of treatments tested) and the number of replicates per experimental group (i.e., statistical significance of results). In an effort to meet both primary and secondary demonstration objectives, the allocation of liquid flow channels in the field demonstrations detailed here balanced both desires and thus featured 3 experimental groups conducted in triplicate, and 2 experimental groups in quadruplicate. (Table 5–1, Table 5–2)

Table 5-1. Experimental plan for NAS North Island (Site 1).

Experimental Group	Replicates	Column Medium	Inoculum	Amendment
Natural Attenuation	3	Site Sediment	-	-
Biostimulation	3	Site Sediment	-	Sodium Lactate
Bioaugmentation	3	Site Sediment	KB-1®	Sodium Lactate
Influent Control	3	-	-	-

Table 5-2. Experimental plan for Site 2.

Experimental Group	Replicates	Column Medium	Inoculum	Amendment
Natural Attenuation	4	Site Sediment	-	-
Bioaugmentation	4	Site Sediment	Microbial Consortium	Sodium Acetate
Influent Control	4	-	-	-

5.2 BASELINE CHARACTERIZATION

5.2.1 NAS North Island - Prior Laboratory Treatability Studies

The following subsection is a brief summary of the relevant laboratory treatability studies investigating *in situ* treatments for OU-20 [refer to the Bench Study Report(SES-TECH 2010) for detailed results and discussions]:

SiRem was retained to evaluate 5 *in situ* treatments for the Cr(VI) and TCE present at OU-20 in bench-scale batch bottle tests. The slow-release substrate SRS-M (Terra Systems Inc., Wilmington, DE) in conjunction with bioaugmentation culture KB-1® (SiRem Inc., Guelph, Ontario Canada) was identified as the best performing and most cost-effective remediation strategy. Below are the manufacturers' descriptions of the chosen amendments:

- SRS®-M contains a proprietary food grade reductant compound plus 60% soybean oil, food grade emulsifiers, sodium lactate, and organic and inorganic nutrients including nitrogen,

phosphorus, and vitamin B₁₂. Additionally a reductant reacts directly with hexavalent chromium to reduce it to the trivalent state. SRS[®]-M provides a readily degradable carbon (lactate) to rapidly generate reducing conditions and a long-lasting carbon source (soybean oil) to maintain the reducing conditions (according to manufacturer's specifications).

- KB-1[®] is a bioaugmentation culture that contains *Dehalococcoides* (*Dhc*), the only group of microorganisms documented to promote the complete dechlorination of chlorinated ethenes to non-toxic ethene (according to manufacturer's specifications).

A detailed analysis of lab treatability study results can be found in section 6.6.

5.2.2 NAS North Island – Prior Field-Scale Pilot Study

A brief summary of the relevant feasibility study objectives is presented here [refer to the FS Report (SES-TECH 2011) for detailed results and discussions]:

Stated objectives of the field-scale pilot test were:

- Evaluate the capacity of the formation to receive the injected amendments.
- Evaluate the distribution and survivability of injected bioaugmentation cultures.
- Evaluate radius of donor delivery (RODD).
- Evaluate the effectiveness of the donor in reducing concentrations of Cr(VI) and TCE in groundwater.
- Evaluate the potential for contaminant presence in vadose zone soils and effectiveness of the amendment in reducing contaminant levels in soils.

Pilot Study Conclusions

The two injection methods tested - liquid atomized injection and direct-push injection - were both found to be effective at distributing the donor and culture in the aquifer; direct-push injection was chosen as the delivery method based on a cost analysis.

Where amendments were distributed, reductions in Cr(VI) and chlorinated ethene concentration were observed within one to three months. SRS-M and KB-1® injections were recommended for full-scale implementation. See section 6.6 and 6.7 for a detailed comparison between field-scale, bench-top laboratory, and ISMA results.

5.2.3 Industrial Site – Prior Lab Treatability Studies

Geomatrix Consultants, Inc. conducted a laboratory treatability study in 2007 (Geomatrix Consultants 2007) to evaluate the potential of biological perchlorate reduction in the vadose zone at site 2 (vadose zone contains the bulk of perchlorate in the subsurface, as described in 4.4.2). Nine polyethylene columns were filled with soil samples from the WBO area. During the 7-month study the columns were spiked with perchlorate and amended periodically with moisture and different carbon substrates (hexene, sodium acetate, yeast). The columns were incubated under non-saturated, anaerobic conditions. Perchlorate was reduced to varying extends (35-56%) in the columns that received carbon amendments, while the non-amended control column showed 24% reduction in perchlorate (natural attenuation conditions). The reasons for the incomplete perchlorate reduction are likely the low moisture content (7.3 – 8.5%) and low numbers of microorganisms present.

The second part of this laboratory study involved filling open glass containers with perchlorate-contaminated site soil and amending with periodic additions of ethanol, corn syrup, sodium acetate, moisture and/or yeast. The microcosms were incubated under anaerobic conditions and under two moisture contents (20 and 45%). In a second phase, the microcosms were bioaugmented with a microbial culture (ZEP® Septic Cleaner) and anaerobic digester sludge from a wastewater treatment plant as well as a further dose of sodium acetate. Significant perchlorate reduction (>99.98%) was only found in microcosms with 45% moisture content and only after amendment with anaerobic sludge and carbon source, but not in the microcosms with 20% moisture content (with or without bioaugmentation) or control microcosms without any amendment. This supports the finding that the low moisture content and presumably high oxygen tension are inhibiting microbial perchlorate reduction.

Geosyntec Consultants conducted another laboratory batch study through SiREM to assess biodegradability of perchlorate in the currently unsaturated zone (Consultants 2009). They focused on two scenarios which might prove challenging: 1) A zone with perchlorate (hundreds of mg/kg soil) and high ammonium concentration (2200 mg/kg soil – as nitrogen) present; 2) a zone with high concentration of perchlorate (1525 mg/kg soil). Batch microcosms were setup with soil from well WBO-1 soil from these zones, respectively, and deionized water, adding methyl soyate as electron donor to stimulate biodegradation of perchlorate. Details of the experimental setup are listed in Table 5-3.

Table 5-3. Experimental plan for Geosyntec laboratory batch study.

Experimental Group	Replicates	Column Medium	Inoculum	Electron Donor
Control	3	Site sediment	-	-
High Ammonium	3	Soil with perchlorate and 2200 mg/kg ammonium as nitrogen	ZEP in bottle 4	Methyl soyate; ethanol in bottle 4
High Perchlorate	3	Soil with 1525 mg/kg perchlorate	ZEP in bottles 8 + 9	Methyl soyate; ethanol in bottles 8 + 9

Perchlorate degradation in these microcosms was highly variable. In two of the three microcosms that received high-ammonium soil, perchlorate was completely reduced after 30 days. In the third microcosm with that same soil, perchlorate reduction was not observed over the whole observation period of 100+ days, even after subsequent addition of ethanol as additional electron donor and a commercial microbial culture (ZEP® Septic Cleaner).

In two of the three microcosms that received high-perchlorate soil, perchlorate was reduced significantly, although not completely, after more than 100 days of observation. No perchlorate reduction was observed in the third microcosm with high-perchlorate soil, even after addition of ethanol and the commercial culture.

Control microcosms with no electron donor or microbial culture added showed no reduction of perchlorate in all three replicates.

5.2.4 Water Sampling

Prior to deployment of the ISMA, a water sample was retrieved from the deployment well and analyzed for dissolved oxygen (DO), redox potential (ORP) and pH in the field using a pre-calibrated multi-parameter probe (YSI Inc., Yellow Springs, OH). Further, the water sample was analyzed for its concentration of chlorinated ethenes (trichloroethene, *cis*-dichloroethene, vinyl chloride) as well as concentrations of dissolved metals that are relevant for drinking water (arsenic, chromium, iron, manganese, selenium). Samples were handled using proper chain-of-custody procedures and were analyzed by certified commercial laboratories for the demonstration at NAS North Island.

5.3 TREATABILITY OR LABORATORY STUDY RESULTS

5.3.1 NAS North Island - Laboratory Flow-through Experiments

Batch bottle treatability studies conducted by SiRem are summarized in section 5.2.1, and a detailed comparison between those lab results, field pilot-scale, and ISMA results can be found in sections 6.6 and 6.7. The following is a summary of sediment column construction and operation in the laboratory at ASU prior to column deployment *in situ* at NAS-NI.

Column construction: On Aug. 22, 2011, composite sediment from the drilling of multiple wells the previous week at NAS-NI was collected into a 5 gallon bucket and transported back to ASU. In the ASU lab, the sediment was transferred into a shallow tray and allowed to air dry in the fume hood over a period of approximately 3 days. Dried sediment was then sifted to collect particles ranging in size from 1000 to 250 μm in diameter, that were then packed into 9 glass ISMA columns.

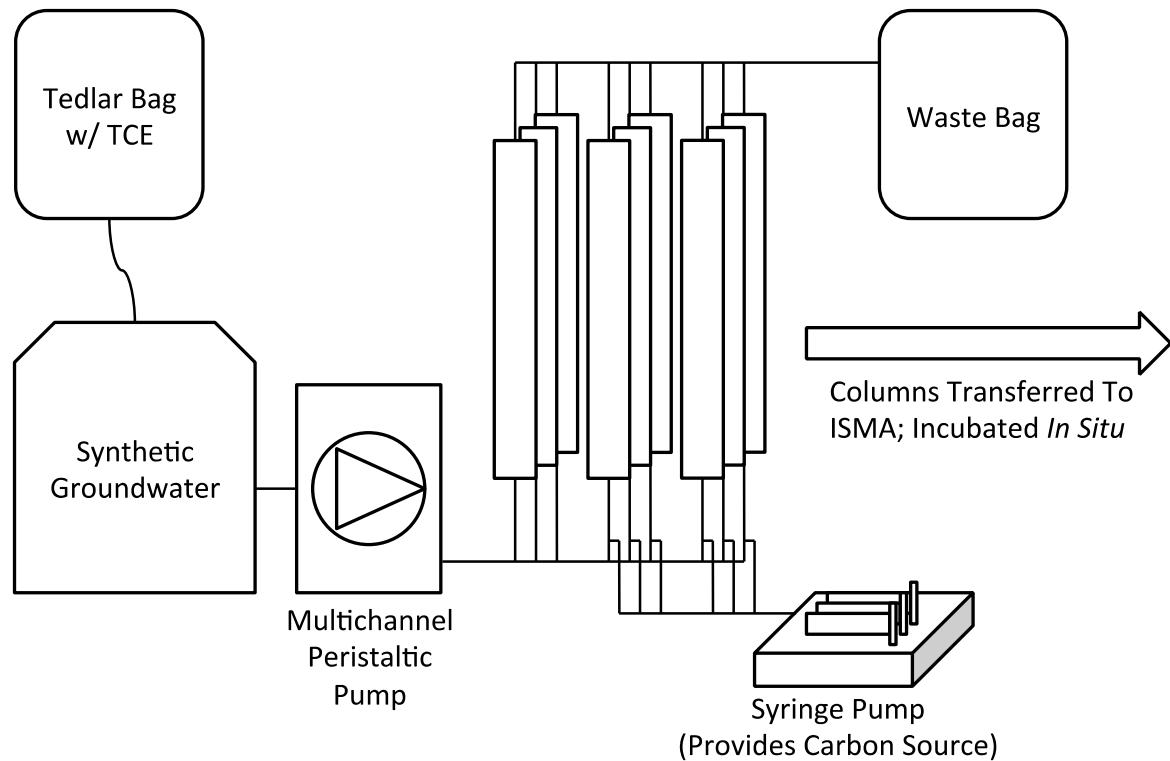


Figure 5-1. Schematic of laboratory column setup.

Column operation and startup: Columns were assembled as shown in Figure 5-1. To ensure a stable TCE concentration in the influent, a Tedlar bag, filled with air already at equilibrium with the headspace in the groundwater bottle was connected to the groundwater bottle so that it supplied the bottle with air as the groundwater was pumped out. Columns were fed with synthetic groundwater (recipe in Appendix B) in a pulsed influent-feed cycle, with the pumps on for 90 seconds at a flow rate of 56 $\mu\text{L}/\text{min}$, followed by a 240 second pause, resulting in an effective flow-rate of 0.91 mL/hour, which translates into a residence time of 10.45 hours and a linear velocity of 1.8 ft/day, assuming a porosity of 0.4.

Column effluent samples were analyzed for chlorinated ethenes and ethane using an automated headspace solid phase microextraction followed by gas chromatography and flame ionization detection method (HS SPME GC-FID) developed in our laboratory that enabled accurate measurements with only 0.2 mL of liquid (Ziv-El, Delgado et al. 2011).

After 5 days, once TCE concentrations in column effluent had stabilized and matched the 15 $\mu\text{g-TCE/L}$ supplied in influent, the three columns comprising the bioaugmentation experimental

group were inoculated w/ KB-1®. Inoculation was carried with a gas-tight syringe by injecting approximately 3 mL of the microbial culture as received from SiRem in a serum bottle at the into the influent (bottom) port of the column. Immediately after inoculation, the influent of the six columns comprising the bioaugmentation and biostimulation experimental groups began to be amended with sodium lactate. The amendment, a 10% w/v sodium lactate solution, was continuously dispensed to each column influent at flowrate of 0.231 μ L/min from an array of six 10 mL plastic syringe powered by the ISMA injection module, resulting in an effective concentration of 50 μ M lactate in each columns influent.

On Day 12, after complete conversion of influent TCE to cDCE was observed in the bioaugmented columns, the columns were reinoculated with KB-1® to ensure presence of viable populations of obligate anaerobes.

Figure 5-4 shows the results for molar fractions of chlorinated ethenes and ethene detected in column effluent. Each graph represents the average of 3 columns. For each graph, mass is normalized to the total molar mass of TCE, cDCE, VC, and ethene collected at that sampling event. On day 75, 70 days after the initial inoculation event, all bioaugmented columns were successfully converting all influent TCE to ethene. In the same timeframe, biostimulated columns were only converting approximately half of influent TCE to ethene, and unamended columns showed no evidence of reductive dechlorination. After 80 days of operation in the laboratory, columns were transferred *in situ* to NAS-NI.

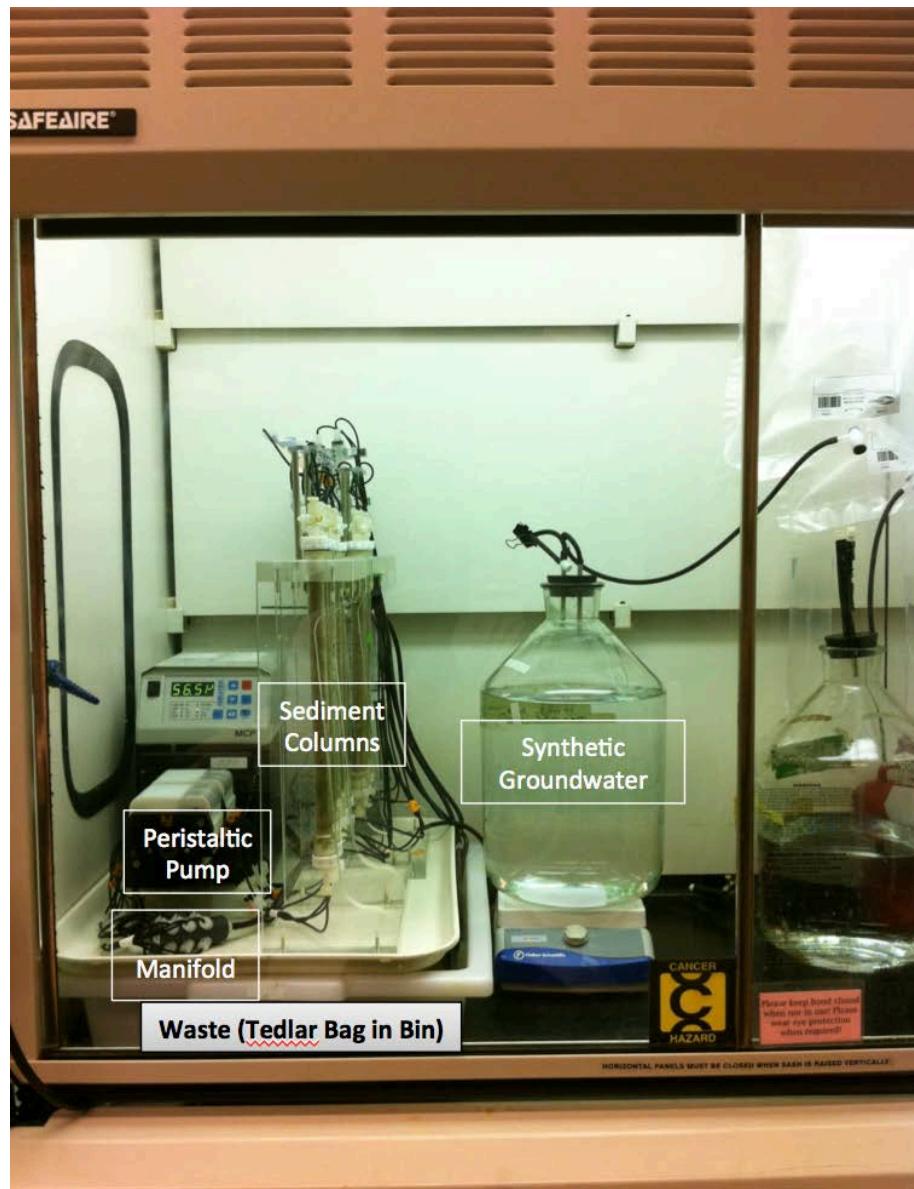


Figure 5-2. Columns filled with sediment from NAS-NI being operated under continuous flow conditions in the laboratory. Entire assembly takes up approximately 5 sq. ft. in a fume hood.

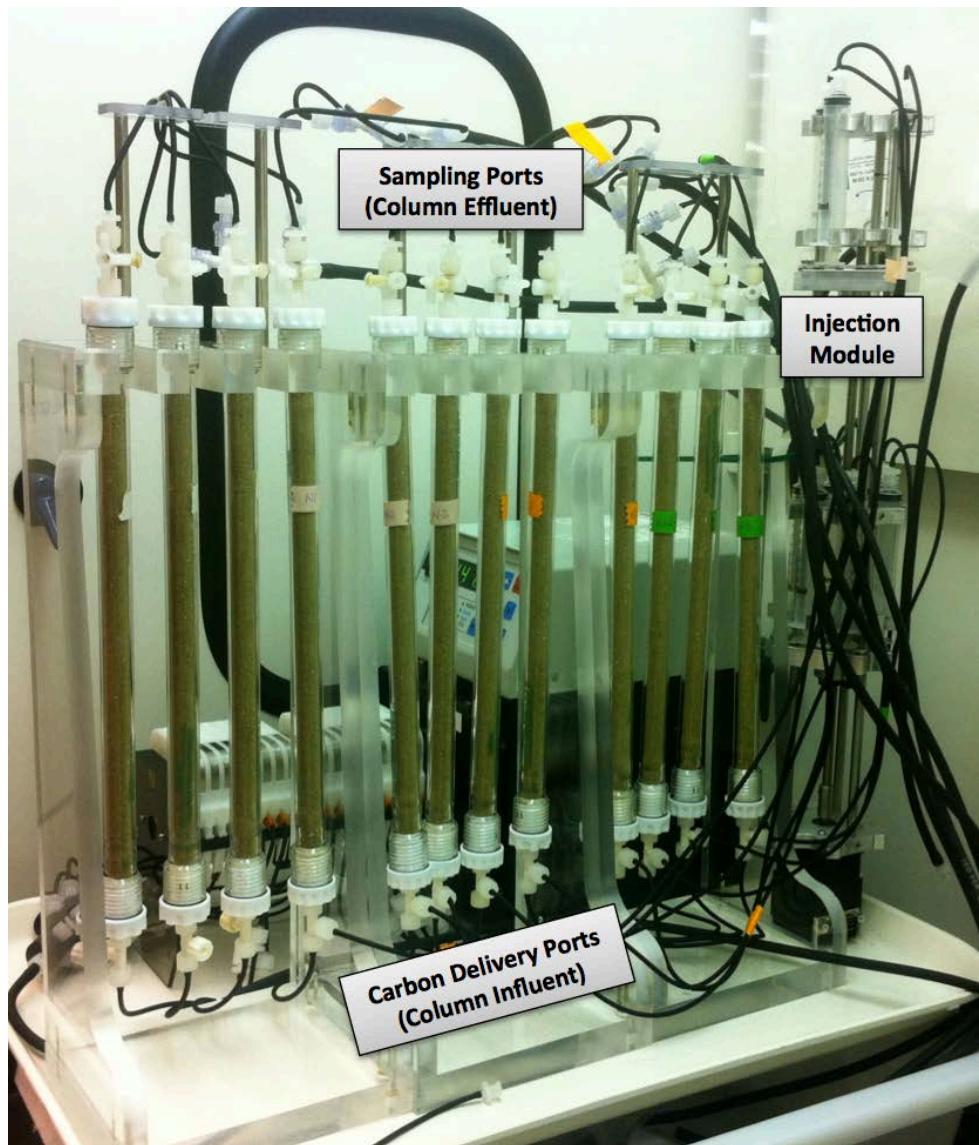


Figure 5-3. Close up view of the sediment columns and the Injection Module shown in Figure 5-2.

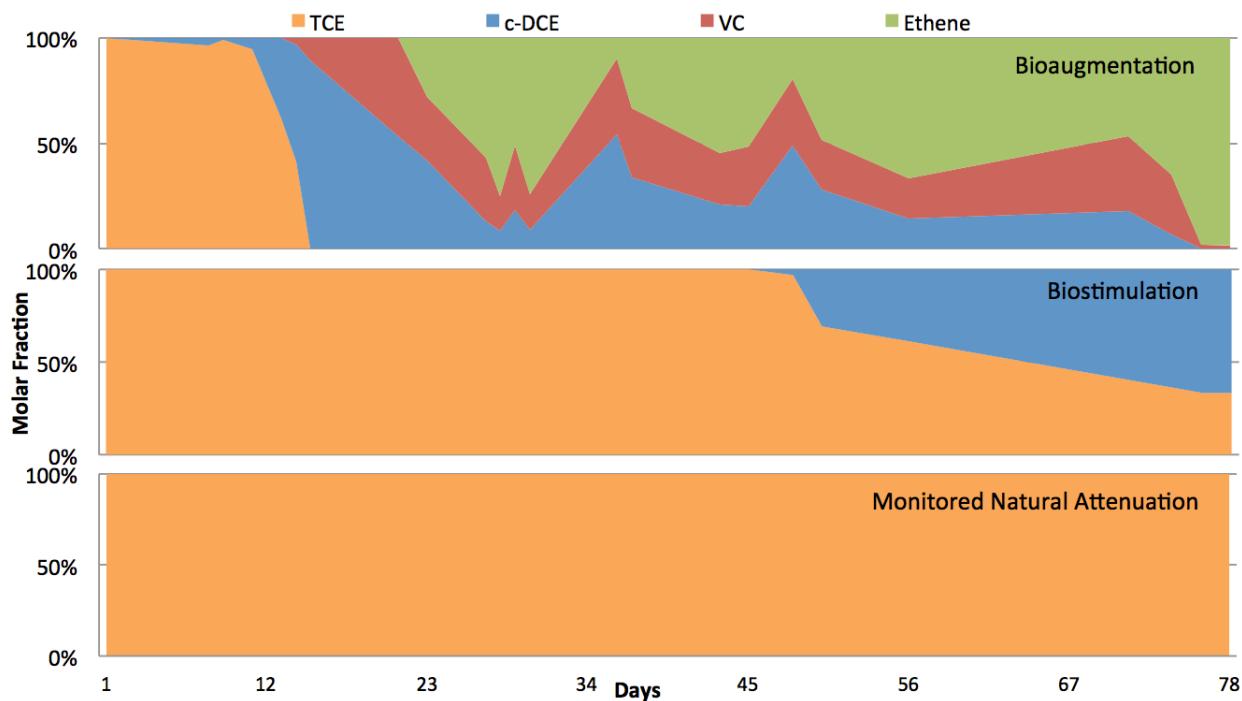


Figure 5-4. Results from column effluent samples.

5.3.2 Industrial Site - Laboratory Experiments in Batch

We were able to reproduce the results from prior laboratory batch experiments (detailed description in section 5.2.3) in our own batch microcosm study with site sediment from well HPA-1. Batch bottle experiments were conducted in 200-mL serum bottles capped with butyl rubber stoppers. Five replicate bottles were filled with 150 mL site groundwater and 5 g dried, well graded, washed sediment (<0.5 mm grain size) from the site. Each bottle was spiked with ethyl lactate (1000 mg/L) and perchlorate (1000 μ g/L). No attempts were made to remove oxygen from the bottles at the beginning of the experiments. However, once capped, bottles were sampled periodically using gas-tight techniques to prevent oxygen from getting into the bottles thereby enabling the development of anoxic conditions through microbial activity. Samples were analyzed for perchlorate concentration.

Results in Figure 5-5 show that it took between 6 and 13 days to achieve complete reduction of 1 mg/L perchlorate by biostimulation with ethyl lactate alone.

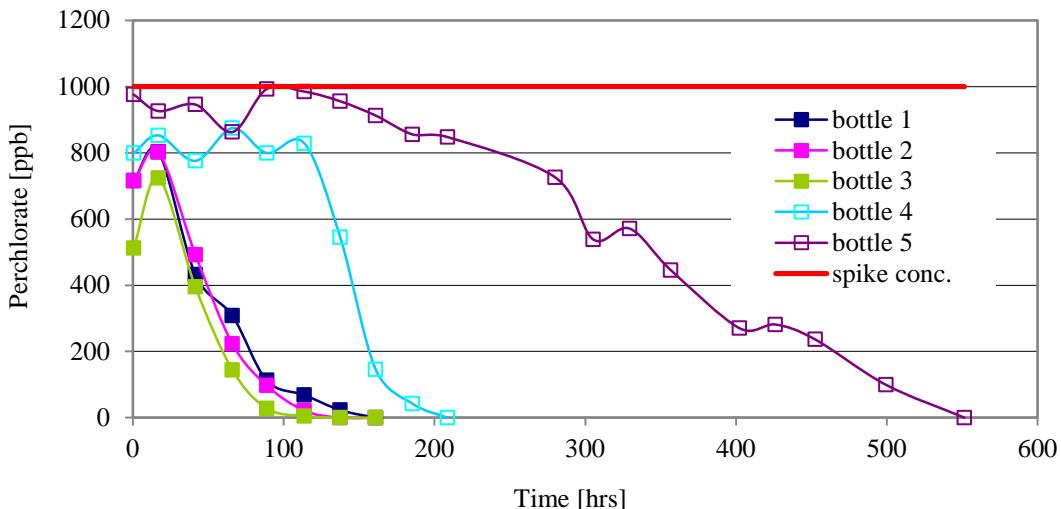


Figure 5-5. Biostimulation of site sediment (well HPA-1) and site groundwater in batch bottles with ethyl lactate as carbon source.

We therefore agree with Geosyntec Consultants conclusion that the native microbial population at the site is very heterogeneous. We consequently chose to use a known perchlorate-reducing inoculum as a bioaugmentation agent at this site.

5.3.3 Industrial Site - Laboratory Experiments in Flow-through Columns

No flow-through column studies had been conducted previously for this Arizona site. We therefore conducted a laboratory column study examining A) natural attenuation (MNA), B) bioaugmentation with perchlorate reducing culture and amendment with ethyl lactate (B1) or sodium acetate (B2). All experiments were conducted in triplicate. As a control, influent groundwater was collected in the same fashion as microcosm effluent over the duration of the experiment without passing through sediment columns. All experiments were conducted simultaneously using the same source of site groundwater.

The seed culture for bioaugmentation experiments, a facultative anaerobic microbial consortium enriched from sewage sludge obtained from five different U.S. wastewater treatment plants, was utilized to accelerate the onset and rates of perchlorate reduction. For the purpose of bioaugmentation, 1 mL of seed culture was added to each bioaugmentation microcosm at the beginning of the experiment by injection of the liquid culture at the influent (bottom) of each column. Sodium acetate trihydrate was added at 1100 mg/L influent concentration in experiment

(B_1), and ethyl lactate at 340 mg/L in experiment (B_2). To compare bioaugmentation to the effects of natural attenuation, three columns were operated without addition of carbon source or biomass (Experiment A). All microcosms were operated in up-flow mode at 15 μ L/min flow, equivalent to residence time of 10 hours in the column.

Microcosms were packed with well graded sediment (0.5 - 1 mm grain size) obtained from drill cuttings from well HPA-1. Site groundwater containing about 500 μ g/L perchlorate was used as the microcosm influent for laboratory flow-through experiments. All lab experiments were conducted at room temperature, which is similar to the groundwater temperature of ~23°C at the deployment site in Arizona.

The effluent of all microcosms was collected as a composite sample throughout the duration of the experiment. Effluent was stored at room temperature in individual Teflon® vessels containing a microbial preservative (Kathon®, minimum concentration 0.5 mL/L effluent). In addition, time discrete samples of the effluent were collected periodically, filtered through a 0.45 μ m polyvinylidene difluoride (PVDF) filter (PALL Life Sciences, Port Washington, NY), and analyzed for pH as well as concentration of perchlorate, nitrate, nitrite and sulfate using established techniques as described below.

Experiments were conducted for a period of 3 weeks. After termination of the experiments, composite effluent samples were analyzed for the same parameters as time-discrete samples. In addition, DNA was extracted from microcosm effluent as well as the column sediment.

After bioaugmentation with a seed culture and both carbon amendments (experiment B_1 and B_2), perchlorate was reduced consistently after an adaptation period of two days (Figure 5-6), while monitored natural attenuation (MNA - experiment A) did not lead to perchlorate reduction over the course of the experiment.

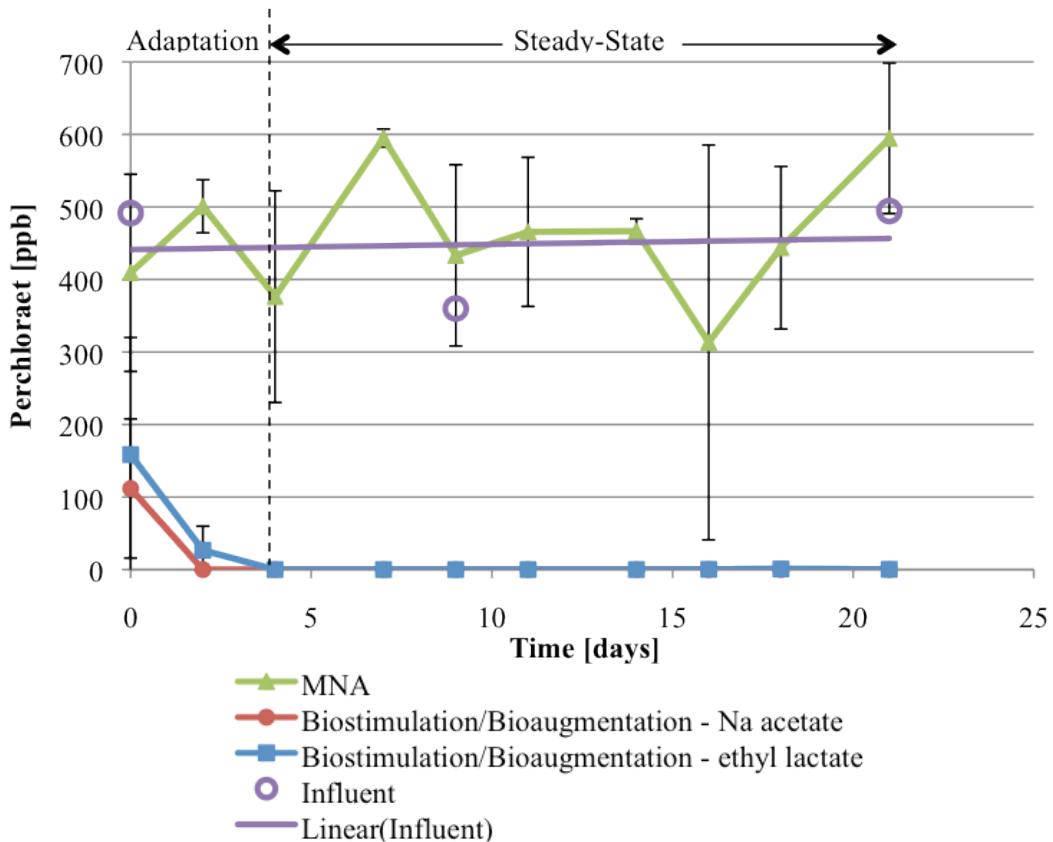


Figure 5-6. Concentration of perchlorate in column effluent over the course of the experiment. All experiments were conducted in triplicate, except influent concentration is measured from one sample at a time. Error bars represent standard deviation.

The groundwater also contained around 5 mg/L sulfate and a very low concentration of nitrate (<1 mg/L), both of which could serve as electron acceptors for the microbial community. Nitrate was reduced to <0.01 mg/L for both carbon amendments in less than two days, and no nitrate was detected for the remainder of the experiment. Sulfate was completely reduced to <0.01 mg/L in the microcosms with ethyl lactate amendment (*B2*) after an adaptation period of 16 days. During the adaptation period, sulfate concentrations decreased steadily. In sodium acetate amended microcosms (*B1*) sulfate concentrations started decreasing after 18 days, but only some sulfate was being reduced at the end of the experiment after 21 days. In MNA microcosms (*A*), neither nitrate nor sulfate was reduced throughout the experiment.

While nitrate is typically reduced before the onset of perchlorate reduction (Chaudhuri, O'Connor et al. 2002) or simultaneously with perchlorate reduction (Herman and Frankenberger

1999), the presence of sulfate has not been shown to affect the ability of bacteria to reduce perchlorate. Therefore, reduction of sulfate is not desirable for *in situ* remediation of perchlorate, as it consumes valuable carbon source and may produce hydrogen sulfide, which is toxic to many organisms.

DNA analysis of the column effluent and sediment revealed that sodium acetate stimulated the growth of bacteria and specifically of perchlorate-reducing bacteria much more effectively than ethyl lactate. This was evident from gene copy numbers for 16S rRNA genes and perchlorate reductase (*pcrA*) genes in both effluent and sediment, which were on average 43±74 times higher when sodium acetate rather than ethyl lactate was supplied as a carbon source (Figure 5-8).

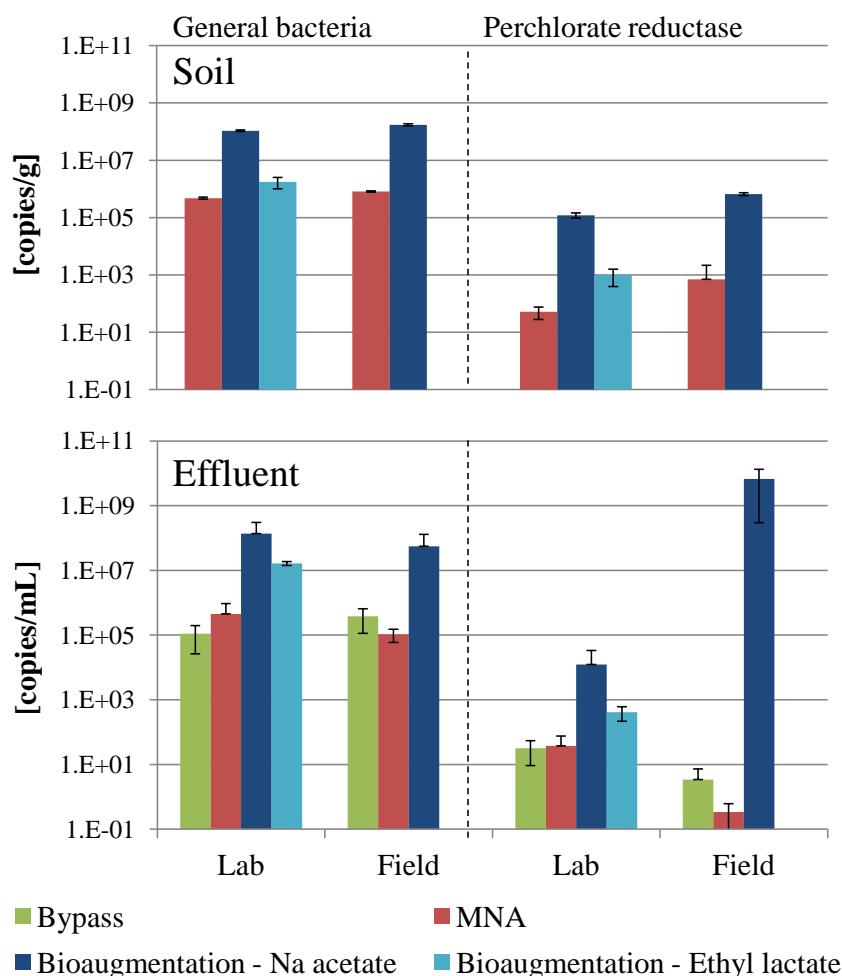


Figure 5-7. Results of quantitative PCR targeting the 16S rRNA gene of general bacteria and perchlorate reductase (*pcrA*). Shown are sediment results for the influent section of each column, which contained the highest numbers of bacteria compared to mid and effluent section. N/A = not tested in field experiment. Error bars represent standard deviation.

5.4 DESIGN AND LAYOUT OF TECHNOLOGY COMPONENTS

5.4.1 Outer Shell of the ISMA

The outer shell of the device and some internal components were designed using computer-aided design software (3DS SolidWorks, Dassault Systèmes SolidWorks Corp, Waltham, MA). To fit within the constraints of common 10-cm (4 in) inner diameter groundwater wells, many components of a standard laboratory column study needed to be miniaturized. Design restrictions included an 8.9-cm outer diameter (OD) of the device, a modular design limiting the length of each module to no more than 2.5 m, and the ability for quick assembly of the device in the field while ensuring reliable functionality of all of its components. All materials needed to be compatible with a range of chemicals potentially extant in contaminated aquifers. Detailed drawings of the different components, which are described below, are provided in Figure 5-9.

5.4.2 Pump Design

Peristaltic pumps were chosen to achieve continuous low flow rates required for simulating slow groundwater movement through sediment microcosms. The pumps needed to supply controllable water flow to multiple channels simultaneously, without potential obstructions in one channel affecting the flow rate in another channel. Additionally, pump hardware could not directly contact contaminated groundwater, as would be the case with other types of pumps (piston pumps, gear pumps). This eliminates the potential for cross-contamination of chemicals between different sites.

An off-the-shelf peristaltic pump (Ismatec; Glattbrugg, Switzerland) was modified to fit within an 8.9 cm outer diameter shell, as required for the ISMA device to fit into a standard 10 cm (4 inch) inner diameter groundwater well. Peristaltic pumps were chosen to achieve continuous low flow rates required for simulating slow groundwater movement through the sediment microcosms. The pump design chosen affords control and uniform flow of water through the multiple parallel channels regardless of differences in conductivity and headloss across the various microcosms. Additionally, none of the reusable parts of the pump hardware can come into contact with contaminated groundwater with the chosen design. Selection of other types of

pumps (piston pumps, gear pumps) would have increased the risk of chemical and bacterial cross-contamination when sequentially using the tool in different wells or at different sites.

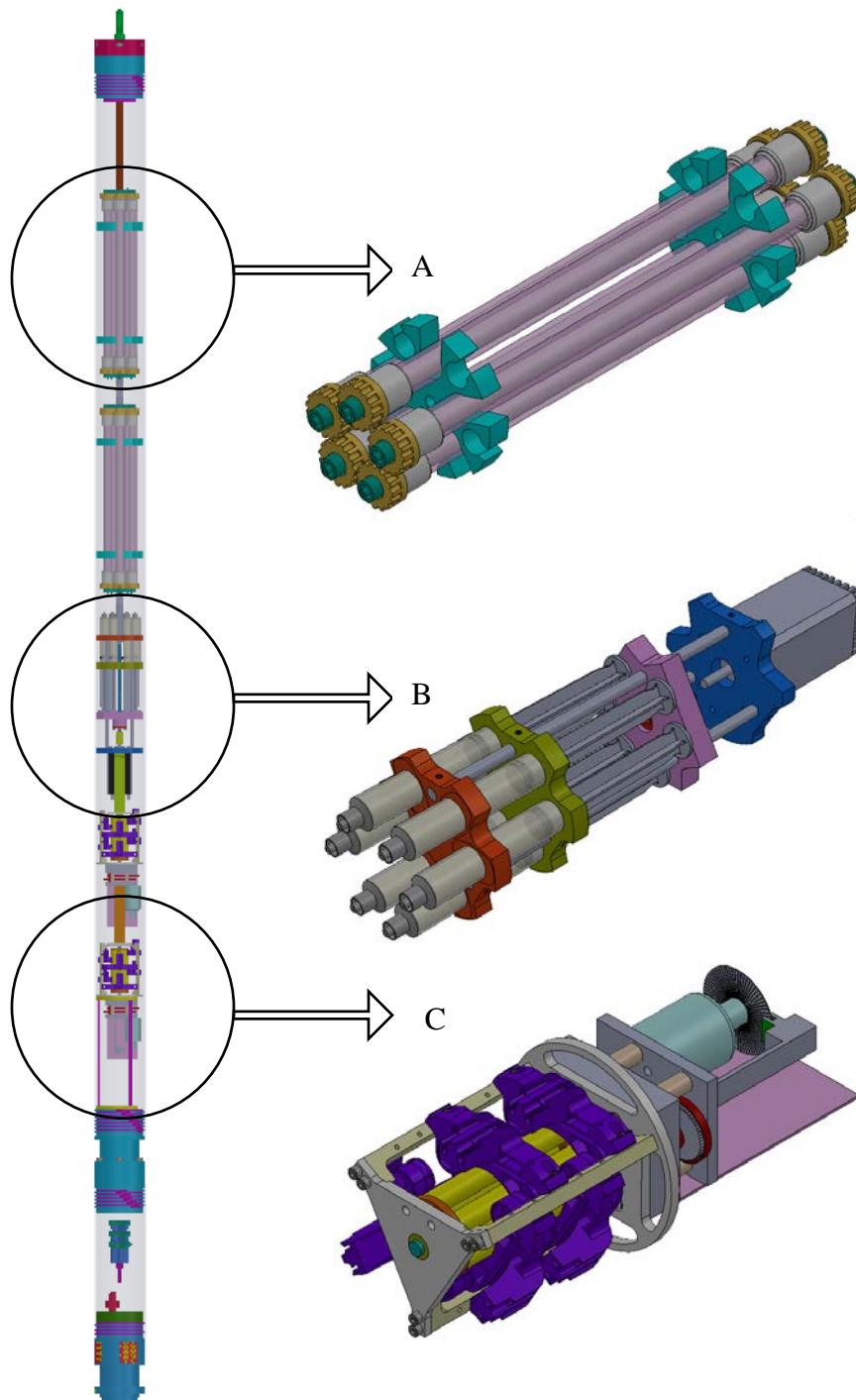


Figure 5-8. Detailed schematic of the ISMA device; Detail A - microcosm array; Detail B - injection module; Detail C - peristaltic pump.

Customizations included re-design of the motor mounting plate as well as the cassettes holding the pump tubing. Pump cassettes that control flow in the pump tubing of the peristaltic pump were manufactured using rapid prototyping technology. The cassette material was chosen for its low surface friction to eliminate rubbing of the tubing material, as well as its rigidity to provide even pressure across the pump tubing. Drawings of the customized pump assembly are shown in Figure 5-9 Detail C.

Performance of the customized pumps was evaluated for long-term stability of delivered flow, accuracy, and inter-channel reproducibility of the flow volume. To test accuracy and inter-channel reproducibility, pumps were mounted in the laboratory and performance tests conducted in triplicate for 4.5 – 5 hrs at flow rates set to 20, 50, 100, and 200 $\mu\text{L}/\text{min}$, respectively. Effluent was collected and measured volumetrically to infer flow rates.

Pump accuracy was also tested for an unmodified comparable pump (Ismatec Reglo Digital, Ismatec, Glattbrugg, Switzerland). The pump was operated in the laboratory with 24 channels at a target flow rate of 79.1 $\mu\text{L}/\text{min}$ in duplicate experiments for 0.5 and 2.7 hours, respectively. Results were averaged over all 24 channels and both tests.

Results shown in Figure 5-11 panels A and B demonstrate that flow rates are accurate (<30% standard deviation) and reproducible between multiple channels over a range of 20 – 200 $\mu\text{L}/\text{min}$ flow.

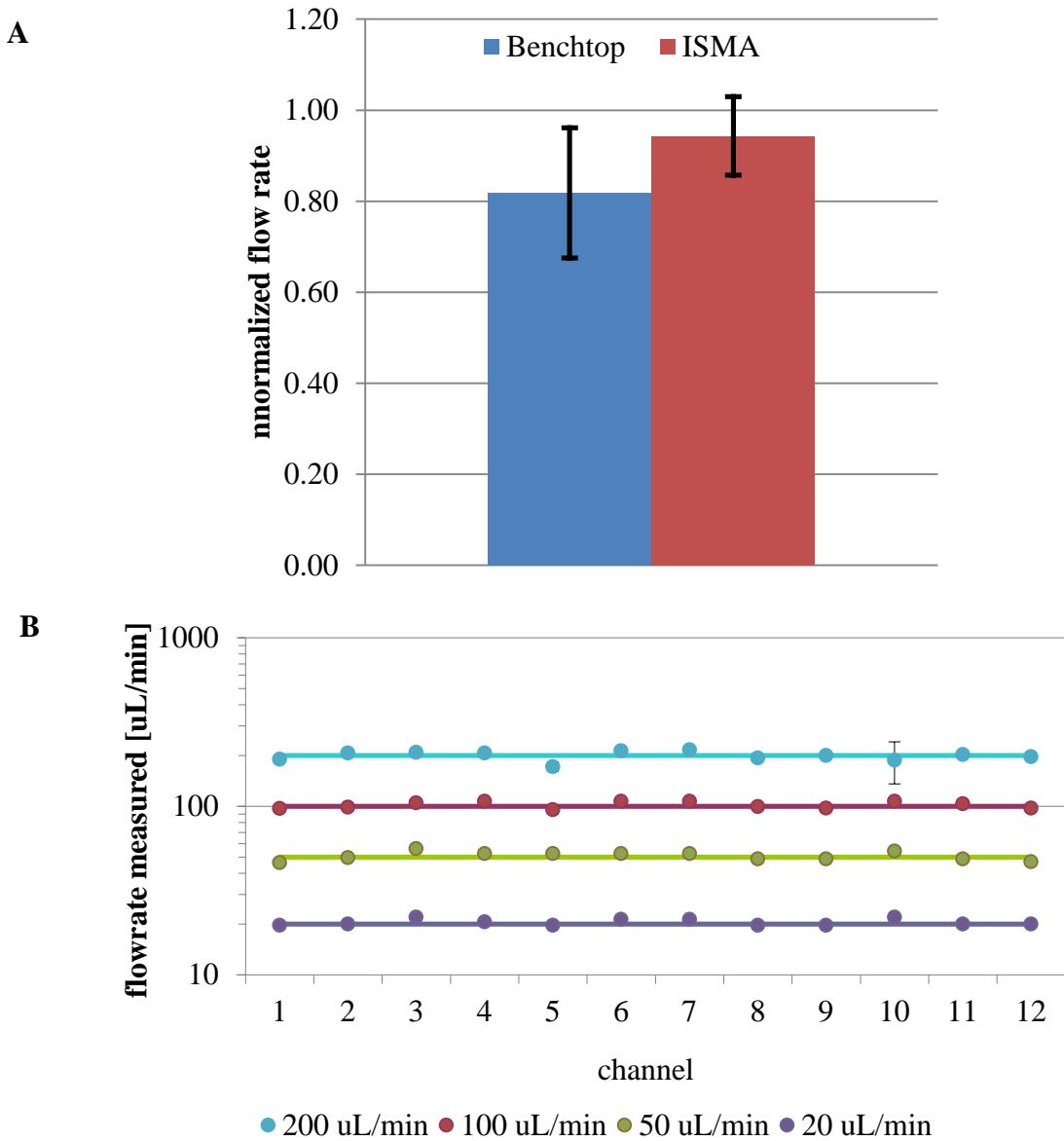


Figure 5-9. Performance control experiments: A - Pump flow rate accuracy for peristaltic benchtop pump (Ismatec Reglo Digital) and customized peristaltic pump used inside the *in situ* microcosm array (ISMA). Tests were conducted for 24 or 12 channels, respectively. B - Flow rate reproducibility between 12 channels for customized pump in the ISMA. Flow rates [$\mu\text{L}/\text{min}$] were set to 20 (Δ), 50 (\times), 100 (\square) and 200 (\circ) as indicated by the solid lines. Shown is the average of three measurements.

5.4.3 Sediment Column Tests

The ISMA contains an array of up to ten flow-through microcosms where a range of treatability experiments can be conducted concurrently. Microcosm vessels consist of custom glass columns (250 mm length, 14 mm ID, Chemglass Life Sciences, Vineland, NJ) with Teflon® screw caps and Viton® o-rings that provide a waterproof seal (Figure 5-9 Detail A).

Microcosms are ideally filled with fresh site sediment where available. If intact cores are not available, archived sediment, representative of the subsurface chemistry where the *in situ* test is to be conducted, can be used. For comparative studies, an alternative stationary phase (quartz sand, activated carbon, sediment mixed with iron filings, etc.) can be used as the packing material in the microcosms.

Six glass columns were packed with dried, well-sorted sediment of two different grain size fractions (each in triplicate) referred to as fine (<0.5 mm) and coarse (0.5 – 1 mm) sediment. Sediment was obtained from a site in Mesa, AZ originating from the Upper Alluvial Unit at 53 m depth. It was characterized as well graded sands, gravelly sands, containing little or no fines, but containing inorganic clays. More specifically, the sediment had a pH of 7.24 and contained 64% sand, 22% silt, 14% clay, 0.06% total organic carbon, 1162 mg/L total Mn, and 18490 mg/L total Fe.

The reproducibility of manually packing the sediment microcosms was tested by injecting a slug of bromide (40 μ L of 5 g/L NaBr) into sediment columns and monitoring effluent bromide concentrations over time. Bromide was analyzed following EPA method 314.0. Details of the analytical method have been previously published (Ahn, Oh et al. 2009). Figure 5-11 shows the tracer curves of two tests using different grain sizes of sediment in the columns. Both tests were carried out in triplicate. The tracer curves show that the replicate columns performed very similarly, proving the reproducibility of the packing method. The data also show that no preferential flow occurred in the columns as indicated by the tracer showing a retention time consistent with the pore volume of the microcosm. Lastly, obtained data show that, as expected, the residence time in the column is dependent on the grain size of the sediment, due to the lower

effective porosity of the smaller vs. the larger grains, which is inversely related to the residence time.

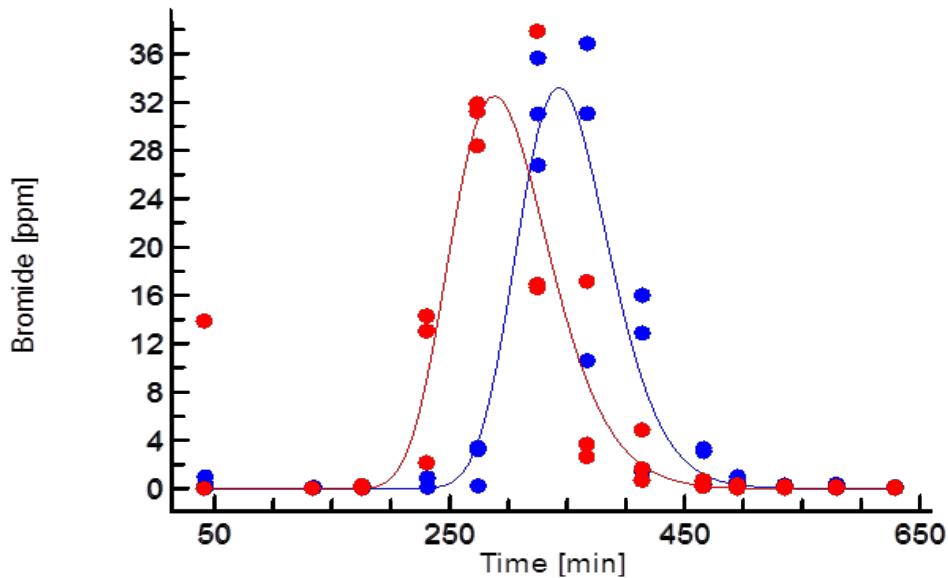


Figure 5-10. Conservative tracer curves showing the bromide concentration in column effluent over time after a one-time injection of bromide. Sediment columns were filled with fine (<0.5 mm – blue curve) or coarse (0.5 – 1 mm – red curve) grains. Experiments were conducted in triplicate.

5.4.4 Delivery of Treatment Agent

To deliver a treatment agent (e.g., chemical or biological agent) to the column microcosms the ISMA device contains a customized syringe pump as an injection module (Figure 5-9 Detail B), which dispenses multiple syringes with a single drive shaft. Different agents can be supplied to each microcosm. The pump rate and concentration of the amendment can be adjusted to simulate different dosing regimens or treatment approaches. Relevant treatment agents can be for example, a carbon source or electron donor to simulate biostimulation at the field scale, active biomass for bioaugmentation, or a chemical oxidizer or reducing agent to simulate *in situ* chemical treatment.

5.4.5 Effluent Capturing

The ISMA device is completely self-contained, which guarantees no impact on the well where the treatability test is conducted. All groundwater pumped through microcosms as well as an influent control (untreated groundwater) is stored inside the device in custom-made Teflon® sample capture vessels (Figure 2-1). To ensure that the degradation activity occurred in the column microcosms, these vessels are loaded with a preservative/quenching agent designed to stop all unwanted biological or chemical activity once the effluent enters the sample capture vessel. Design criteria for the microbial preservative were that it needed to be fairly benign to humans upon accidental contact and provide broad-spectrum inhibition of bacteria, fungi, and yeast. The preservative chosen (Kathon® CG/ICP) contains 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one as active ingredients. It is very stable, compatible with most analyses and is frequently used for abiotic control experiments (Ruiz-Aguilar, Fernandez-Sanchez et al. 2002; Fernandez-Sanchez, Sawvel et al. 2004; Oh and Alvarez 2004; Da Silva, Ruiz-Aguilar et al. 2005; Kazy, Monier et al. 2010). The mixing effectiveness of the preservative with the column effluent was tested using fresh groundwater that was pumped into four sample capture vessels at a rate of 136 $\mu\text{L}/\text{min}$. The preservative was added according to manufacturer's specifications at 0.01% final concentration to two of the vessels, while the other two served as a control with no added preservative. After 24 hours of pumping at room temperature, 100 μL of each effluent was plated in multiple dilutions onto Luria-Bertani agar plates. Plating was done in triplicate. Colonies formed on the plates were counted after incubating them at room temperature for two days. Results reveal that the preservative and groundwater is efficiently mixed, inhibiting microbial growth (Figure 5-10), and therefore further contaminant degradation.

Through microbial activity or chemical reactions, significant amounts of gas (e.g., CO_2 , N_2 , H_2S) can be produced in the column microcosms. The gas is allowed to vent from the sample capture vessels through vent lines, after passing through sorbent cartridges designed to capture volatile analytes. The loss of chemical mass of compounds of interest in escaped gas can thus be inferred from analysis of the sorptive material inside the cartridge.

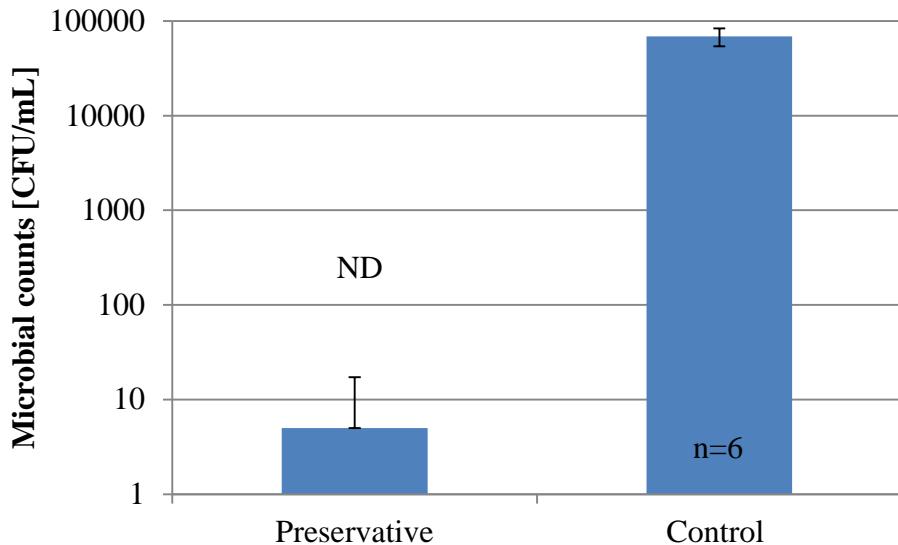


Figure 5-11. Results from preservative test showing plate counts of column effluent treated with preservative and without (control). Experiments were conducted in duplicate; plating was done in triplicate. ND = non-detect (<300 CFU/mL).

5.4.6 Handling of ISMA device

The ISMA is a modular device with three sections, each weighing about 75 lb. Section 1 contains two peristaltic pumps, injection module, and column microcosms array. Sections 2 and 3 contain six effluent compartments each, dedicated to the individual columns and influent lines. The sections are handled by a boom truck for lifting the device and lowering into the deployment well one-by-one (Figure 5-9). In the well each section is secured using a custom clamp (Figure 5-13), and multi-channel quick connectors are used to propagate all fluid and electrical lines (Figure 5-14). Load-bearing, waterproof connections are made between the different sections providing secondary containment. The device is then suspended on a steel cable and lowered to the desired depth.



Figure 5-12. ISMA section being handled by field worker and lowered into deployment well using a boom truck.



Figure 5-13. Securing clamp designed for use with the ISMA device. Left: top view; Right: side view.



Figure 5-14. Left: pre-assembled sections equipped with multi-channel quick connectors for fluid and electrical lines; Middle: connection of multi-channel quick in the field; Right: Load-bearing, waterproof connections are made between the different sections.

5.4.7 Power supply

The ISMA contains two peristaltic pumps and one step-motor that require power. The specific requirements are listed in Table 5-4.

Table 5-4. Power requirements of ISMA components and power provided by battery setup.

ISMA Component	Power requirement			Power provided			
	Voltage [V]	Current [A]	Power [W]	Voltage [V]	Current [A]	Power [W]	El. Charge [Ah]
Peristaltic pumps (2) for Groundwater Delivery	100-230 (AC)	0.363	40				
Step-motor (1) for Injection Module	12	0.1-2	24				
Battery (1)				13 (DC)	1125	13,500	75
Battery (1) plus Inverter				115 (AC)	3.5	400	

The ISMA device may be operated using grid power if available (110V, 50Hz AC). In more remote locations the ISMA can be powered through an array of 12V (DC) batteries that can be combined with multiple solar panels for recharging of the batteries. Both are stored in the direct vicinity of the deployment well.



Figure 5-15. Solar panels and battery array used to provide electrical power for the ISMA device during deployment in remote locations.

5.4.8 Control during operation

The device is controlled through a tether that connects the deployed ISMA in the well to an above ground control unit. The control unit is housed in a weatherproof enclosure. This allows for full control of the pumps and injection motor within the ISMA as well as monitoring of pump performance from the ground surface.

5.5 FIELD TESTING

Before the deployment of the ISMA device, the depth to groundwater was determined and a groundwater sample was retrieved. The individual sections of the device were pre-assembled prior to field-testing. The ISMA was deployed following the procedures described in 2.1 and 5.4.6.

The incubation period was 35 days at NAS North Island and three weeks and at site 2. At NAS North Island, grid power (110V) were used to power the device, while at site 2 a combination of batteries and solar panels was used (see 5.4.7).

After the incubation period the ISMA device was retrieved from the well. Effluent samples and sediment columns were retrieved from the device and stored until analysis or sent to the analytical lab.

Table 5-5. Schedule of deployment activities.

Project start: 05/01/2009	2010				2011				2012	
	Tasks	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
Column Pre-conditioning for NAS-NI						X	X	X		
Deployment of ISMA at NAS-NI									X	
Incubation at NAS-NI									X	X
Retrieval of ISMA at NAS-NI										X
Deployment of ISMA at Site 2							X			
Incubation at Site 2							X			
Retrieval of ISMA at Site 2							X			

5.6 SAMPLING METHODS

Table 5-6. NAS North Island - Sampling Plan.

Component	Sample Collected	# of Samples	Sample Volume	Analytes	Comments
Prior to deployment	Groundwater from deployment well	1	1L	TCE, c-DCE, VC, TDS, drinking water metals, inorganic anions	To assess groundwater quality before deployment
During deployment of ISMA	Untreated groundwater	3	0.75L	TCE, c-DCE, VC, TDS, drinking water metals, inorganic anions	To assess groundwater quality during deployment
During deployment of ISMA	Groundwater flowing through sediment column without amendment	3	0.75L	TCE, c-DCE, VC, TDS, drinking water metals, inorganic anions	To assess potential for Monitored Natural Attenuation
During deployment of ISMA	Groundwater flowing through sediment column with amendment 1	3	0.75L	TCE, c-DCE, VC, TDS, drinking water metals, inorganic anions	To assess treatment potential of amendment 1
During deployment of ISMA	Groundwater flowing through sediment column with amendment 2	3	0.75L	TCE, c-DCE, VC, TDS, drinking water metals, inorganic anions	To assess treatment potential of amendment 2
After deployment	Groundwater from deployment well	1	1L	TCE, c-DCE, VC, TDS, drinking water metals, inorganic anions	To assess groundwater quality after deployment

Table 5-7. Industrial Site - Sampling Plan

Component	Sample Collected	# of Samples	Sample Volume	Analytes	Comments
Prior to deployment	Groundwater from deployment well	1	1L	Major anions + cations, perchlorate, pH, DNA, protein	To assess groundwater quality before deployment
During deployment of ISMA	Untreated groundwater	4	~0.5L	Major anions + cations, perchlorate, pH, DNA, protein	To assess groundwater quality during deployment
During deployment of ISMA	Groundwater flowing through sediment column without amendment	4	~0.5L	Major anions + cations, perchlorate, pH, DNA, protein	To assess potential for Monitored Natural Attenuation
During deployment of ISMA	Groundwater flowing through sediment column with amendment	4	~0.5L	Major anions + cations, perchlorate, pH, DNA, protein	To assess treatment potential of amendment
After deployment	Groundwater from deployment well	1	1L	Major anions + cations, perchlorate, pH, DNA, protein	To assess groundwater quality after deployment

Table 5-8. NAS North Island - Analytical Methods.

Matrix	Analyte	Method	Container	Preservative	Analytical Laboratory	Holding time
Groundwater	Trichloroethylene, cis-1,2-Dichloroethene, Vinyl Chloride	EPA Method 524.2 (Capillary Column GC/MS)	Gastight glass bottle	Maleic acid (0.625 g/L); Ascorbic acid (5g/L)	Columbia Analytical Services, Phoenix, AZ	14 days
Groundwater	Total Dissolved Solids	Standard Methods, Section 2540C	HDPE bottle	As required	Columbia Analytical Services, Phoenix, AZ	1.1
Groundwater	Drinking Water Metals (As, Mn, Cr, Fe, ...)	EPA Method 200.7 (ICP/AES)	HDPE bottle	Nitric acid (pH 2)	Columbia Analytical Services, Phoenix, AZ	6 months
Groundwater	Inorganic Anions (Cl ⁻ , SO ₄ ²⁻ , NO ₃ ²⁻ , NO ₂ ²⁻)	EPA Method 300.1 (IC)	HDPE bottle	As required	Columbia Analytical Services, Kelso, WA	48 hrs

Table 5-9. Industrial Site – Analytical Methods.

Matrix	Analyte	Method	Container	Preservative	Analytical Laboratory
Groundwater	Inorganic Anions (Br ⁻ , F, Cl ⁻ , SO ₄ ²⁻ , NO ₃ ⁻ , NO ₂ ⁻)	EPA Method 300.1 (IC)	HDPE bottle	None	ASU internal
Groundwater	Inorganic Cations (Li ⁺ , K ⁺ , Na ⁺ , NH ₄ ⁺ , Mg ²⁺ , Ca ²⁺)	ASTM Method D6919 (IC)	HDPE bottle	none	ASU internal
Groundwater	Perchlorate	EPA Method 314.1 (IC)	HDPE bottle	none	ASU internal
Groundwater (DNA extract)	DNA	Absorbance at 260 nm wavelength	HDPE bottle	none	ASU internal
Groundwater	Protein	BCA assay	HDPE bottle	none	ASU internal
Groundwater	pH	pH electrode	HDPE bottle	none	ASU internal
Groundwater / Sediment (DNA extract)	DNA	Quantitative PCR	HDPE bottle	none	ASU internal

5.6.1 DATA ANALYSIS

Mass balances are calculated by comparing the chemical mass of interest in the untreated groundwater and the groundwater that passed through columns representing different treatments. Since all experiments are conducted at least in triplicate a mean and standard deviation can be calculated for each data point. Variability between replicates can originate from variability of analytical methods, including extraction, as well as from biological factors such as differences in bacterial growth in different sediment columns. To determine statistically significant differences between treatments as well as between treatment and non-treated control, a student-*t*-test can be conducted for all relevant datasets.

Biological degradation processes generally follow Monod kinetics (Monod 1949) that describe the utilization of a single rate-limiting substrate (the contaminant) and resulting microbial growth. Monod kinetics is characterized by linear degradation of high concentrations of the rate-limiting substrate. This region can be described by a zero-order approximation and is valid for substrate concentrations at least 10 times larger than the half-saturation constant (K_S) of that substrate. For substrate concentrations at least 10 times smaller than K_S , Monod kinetics resembles an exponential function, which can be described by a first-order approximation. First-order kinetics is characterized by a linear profile of the natural log transformed concentration data versus time.

The first-order degradation rate of a contaminant can be calculated for flow-through experiments in the lab, where time-discrete monitoring of the column effluent provide time-resolved data. First-order degradation rate constant ($k_{Discrete}$) are determined using the log transformed contaminant concentration of the influent (C_{in}) and effluent (C_{out}) for each experiment, as well as the residence time in the sediment column (RT).

$$k_{Discrete} = \frac{\ln(C_{in}) - \ln(C_{out})}{\Delta T_{Column}} \quad (1)$$

The first-order degradation rate R is then calculated according to the following equation:

$$R_{Discrete} = k_{Discrete} * C_i \quad (2)$$

where C_i is the mean contaminant concentration.

For ISMA experiments where time-discrete samples were unavailable, a composite sample collected over the duration of the experiment serves to calculate a time-averaged first-order degradation rate.

$$R_{Composite} = k_{Composite} * C_i \quad (3)$$

$$k_{Composite} = \frac{\ln(C_{i_{Influent}}) - \ln(C_{i_{Effluent}})}{\Delta T_{Column}} \quad (4)$$

$R_{Composite}$ is the composite degradation rate, $C_{Influent}$ and $C_{Effluent}$ are the composite perchlorate concentration in the influent and effluent of each column, respectively.

5.7 SAMPLING RESULTS

5.7.1 NAS North Island – ISMA Results

The ISMA was incubated in well OU20-PEW-01 at NAS-NI (Figure 4-4) for 35 days. During the incubation period the pumps operated in a pulsed mode analogous to operation in the laboratory: pumping for 90 seconds at a flow rate of 69.2 $\mu\text{L}/\text{min}$ (as calibrated in the laboratory), pausing for 284 seconds. Target net flow rate was 16.6 $\mu\text{L}/\text{min}$ with a target collected effluent volume of 840 mL and a target column residence time of 9.54 hours. Actual volumes collected were 20% lower than targeted, with an average and standard deviation of 665.5 ± 57.4 mL (greater detail in Table 5–10). The discrepancy between targeted and collected volumes suggests that lab calibration procedure failed to account for the backpressure pumps experienced when the ISMA was fully assembled. However, simulating the full hydraulic head differential in the laboratory for pump calibration is not practical - future deployments should benefit from the empirically derived 20% correction factor when calibrating pumps.

Table 5-10. Groundwater collected during ISMA incubation. Column parameters of residence time, groundwater linear velocities, and pore volumes exchanged assume a porosity value of 0.4.

Channel #	Bypass			MNA			Biostimulation			Bioaugmentation		
	1	2	3	4	5	6	7	8	9	10	11	12
Volume collected	644.0	745.4	621.6	559.0	681.8	593.0	681.1	713.3	751.2	680.0	701.3	614.0
Effective flowrate (ul/min)	12.78	14.79	12.33	11.09	13.53	11.77	13.51	14.15	14.90	13.49	13.91	12.18
Column residence time (hours)				14.28	11.71	13.46	11.72	11.19	10.63	11.74	11.38	13.00
Average Linear Velocity (ft/day)				1.38	1.68	1.46	1.68	1.76	1.85	1.68	1.73	1.51
Pore volumes exchanged				58.82	71.74	62.40	71.67	75.06	79.05	71.55	73.80	64.61

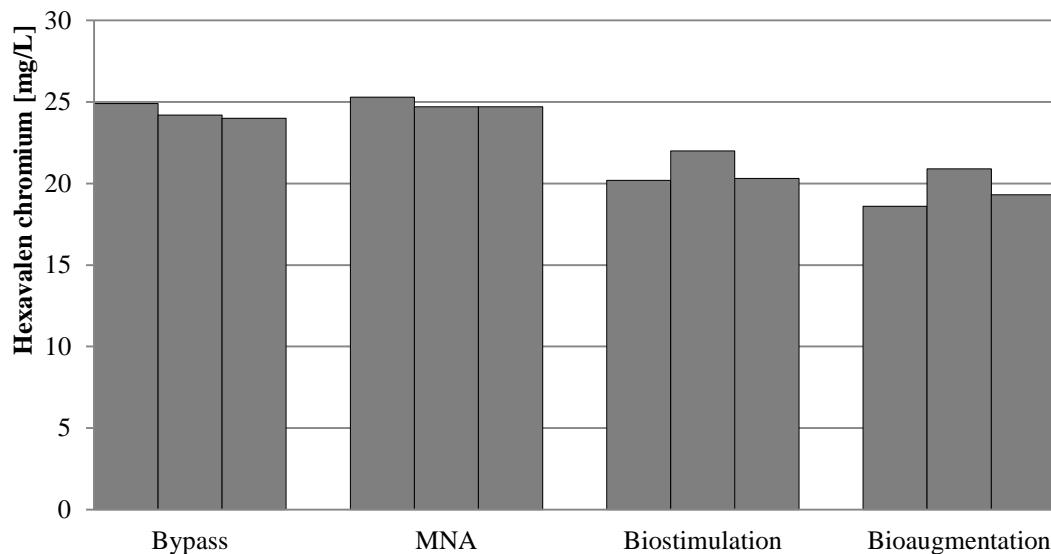


Figure 5-16. Hexavalent Chromium detected in ISMA effluent post *in situ* incubation.

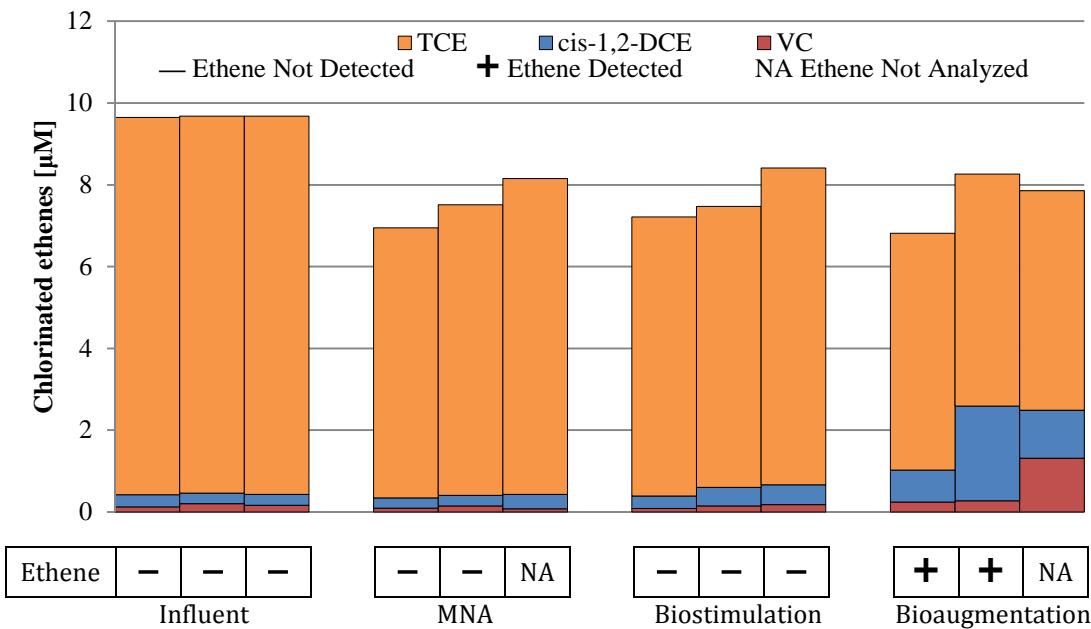


Figure 5-17. Chlorinated ethenes and ethene detected in ISMA effluent post *in situ* incubation.

After ISMA retrieval, collected effluent was subsequently analyzed for hexavalent chromium (Figure 5-16) as well as chlorinated ethenes and ethene (Figure 5-17). Relative to the collected influent, no reduction of hexavalent chromium concentrations was observed in MNA effluent, while both biostimulation and bioaugmentation showed approximately 20% lower concentrations in effluent. These results indicate that stimulation with sodium lactate facilitates chromium reduction, but that additional bioaugmentation with KB-1 does not further enhance chromium reduction. These results are consistent with the literature as well as site-specific bench-top batch bottle treatability studies. A detailed comparison of attenuation rates between batch bottles and *in situ* column data is presented in section 6.6.

Chlorinated ethene results showed approximately 20% lower concentrations of TCE in MNA effluent relative to the influent. This difference can be the result of abiotic TCE attenuation processes (Lee and Batchelor 2002), or may be a result of additional mass loss due to volatilization in the additional length of tubing, fittings, and column apparatus that groundwater must traverse in the MNA experimental lines. Effluent from biostimulation columns showed no difference in detected TCE concentration. Slightly elevated *cis*-DCE concentrations were observed in bioaugmentation samples relative to MNA, however the difference was not

statistically significant (homoscedastic 2 tailed student *t*-test, $p=0.1$), and the mass of TCE that may have been lost to biological reduction to *cis*-DCE was smaller than the overall variability in TCE concentrations detected. Effluent from bioaugmentation columns, however, contained significantly reduced concentrations of TCE ($p<0.05$), and elevated levels of cDCE ($p=0.08$), VC ($p<0.05$), relative to MNA.

Quantification of ethene is challenging due to ethene's extremely high volatility and the fact that it does not sorb well to activated carbon or other sorbents. As mentioned in section 2.1, a sorbent cartridge installed in the ISMA assists with capture of volatile organics, but unfortunately, not with ethene. As a result, quantification of ethene is not possible due to the fact that the bulk of any ethene produced will volatilize and escape through the vent line installed in each effluent capture vessel. Nevertheless, liquid effluent was analyzed for any traces of ethene remaining. Ethene was detected in two bioaugmentation effluent samples at levels below the commercial lab's reporting limit of 1.2 $\mu\text{g/L}$ (0.04 μM), but above the detection limit of 0.6 $\mu\text{g/L}$ (0.02 μM). Unfortunately, ethene analysis was not possible for the effluent from the 3rd bioaugmentation column, due to the fact that all sample was consumed for the analysis for chlorinated ethenes, which was given higher priority. However, analysis of column pore water withdrawn from the column post deployment, as described below, indicates that the third bioaugmentation column likely had the highest amounts of ethene produced (Figure 5-18). No ethene was detected in any other column pore water examined after ISMA retrieval.

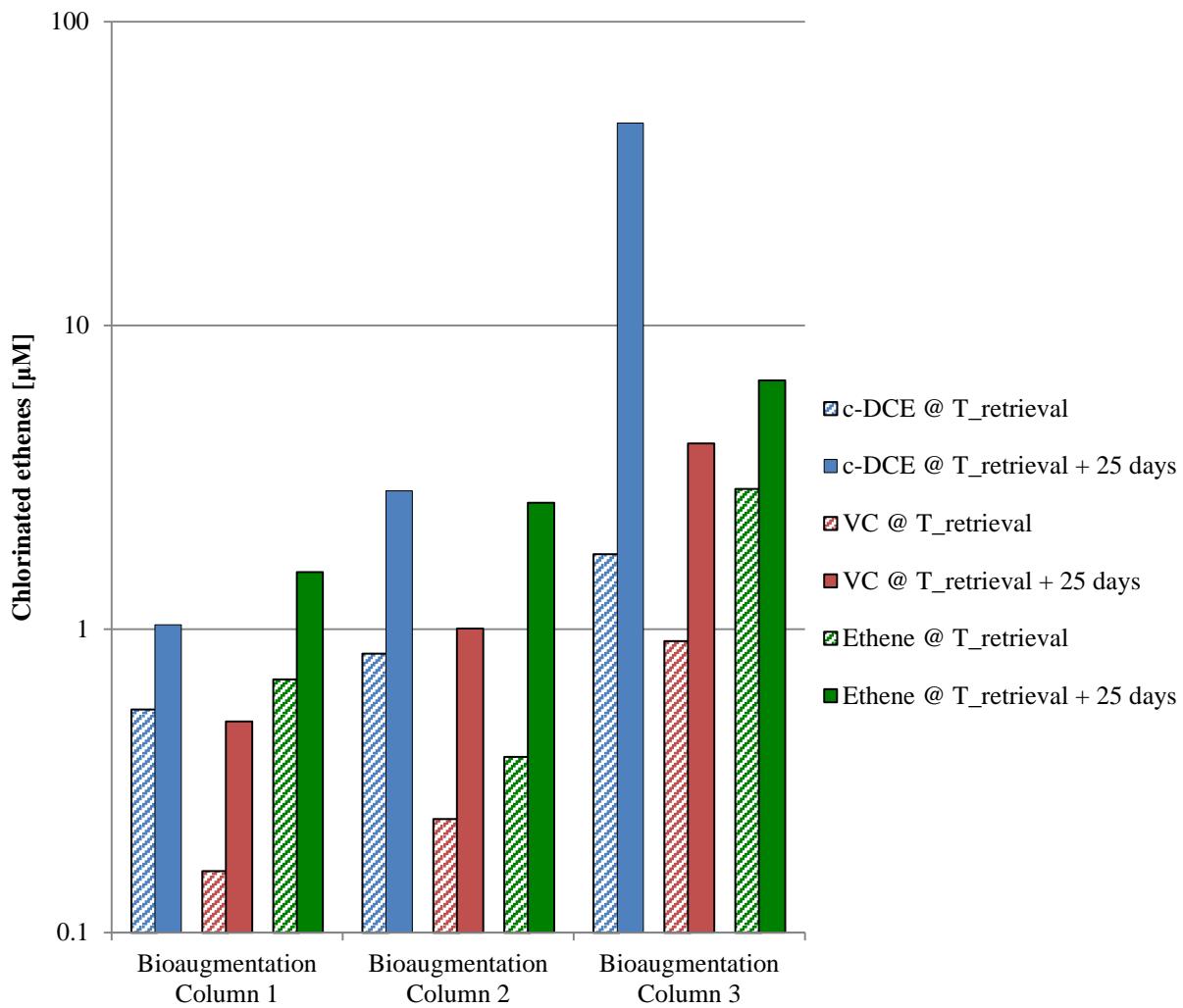


Figure 5-18. Column pore water analysis for chlorinated ethenes and ethene after retrieval and again after 25 days with no flow. Increased concentrations of TCE dechlorination products in all the columns indicate that columns were still biologically active and dechlorinating after *in situ* incubation and exposure to Cr(VI).

Additional work was carried out to unequivocally establish that detected ethene was indeed the product of ongoing biotransformation by the bioaugmented, strict anaerobic microbial community. After ISMA retrieval and transport back to the lab, the sealed sediment columns were incubated in the laboratory without flow at 20 °C, which is equivalent to the temperature of the groundwater *in situ* at the deployment site. The column pore water was then sampled 5 times over a period of 25 days and analyzed for the presence of chlorinated ethenes. Over the sampling period, the bioaugmentation columns showed trends of decreasing TCE concentrations

and increasing VC, *cis*-DCE, and ethene concentrations. The first and last sampling points are presented in Figure 5-18. Results show production of dechlorination products during the post-deployment incubation, indicating that all biological activity in the columns was ongoing after *in situ* incubation.

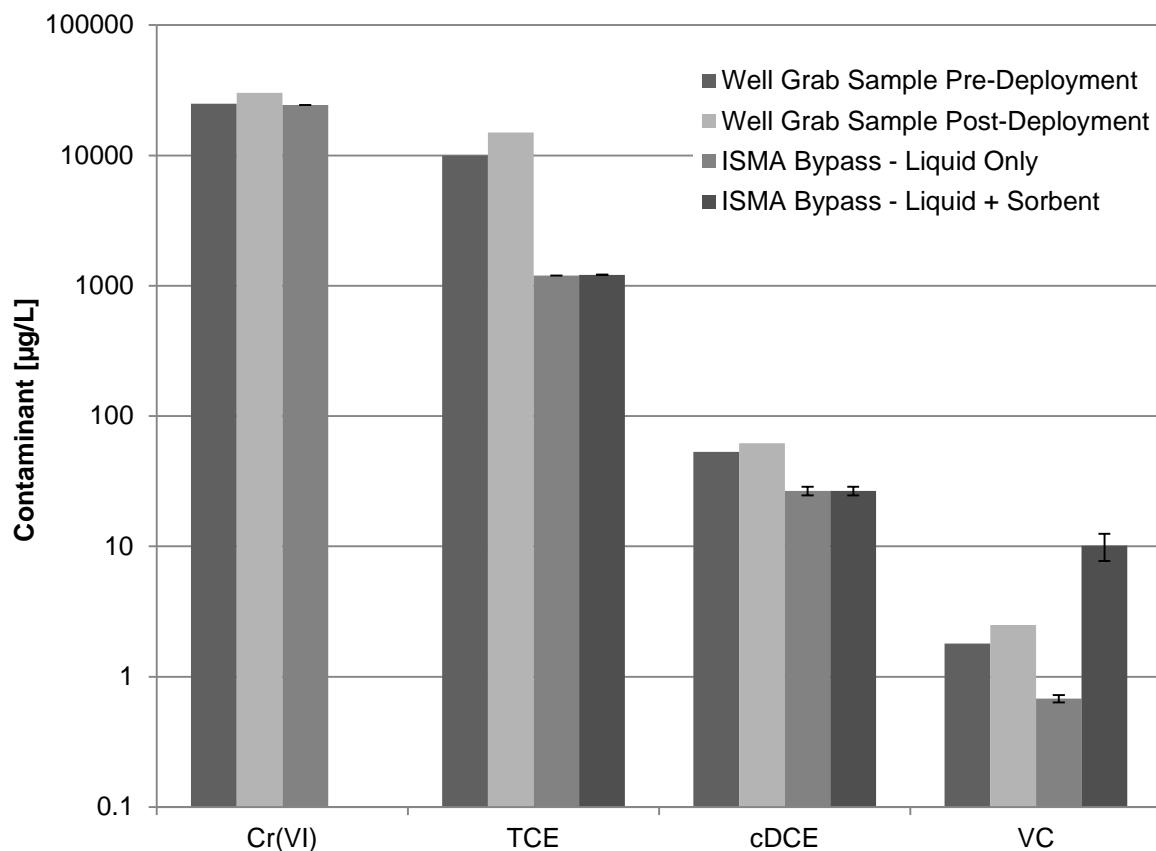


Figure 5-19. Concentrations of primary contaminants detected in the ISMA deployment well compared to those found in ISMA bypass channels.

A comparison is made between concentrations of contaminants detected in the deployment well and in the collected influent in Figure 5-19. The first noteworthy observation is that concentrations in the well fluctuated significantly between deployments. The deployment well is in a tidal zone and as such multiple parameters will fluctuate over time, as shown in Table 5-11. A comparison between ISMA influent and well grab samples demonstrate that the ISMA has excellent capture ability of non-volatile and stable compounds like hexavalent chromium. These results are consistent with other parameters analyzed from the NAS-NI deployment, as well as the deployment from Industrial Site 2. Results shown in Figure 5-19 also demonstrate that

recovery and *in situ* preservation of volatile compounds like TCE is challenging. Concentrations of volatile compounds detected in the influent stored in the ISMA were up to an order of magnitude lower than those detected in well grab samples. These known losses have to be attributed to the extended holding period of groundwater in the effluent capture vessels. This result is supported by the fact that concentrations of chlorinated ethenes detected in groundwater from column pore water were in the same order of magnitude as those found in the groundwater sampled at the site and shipped to the commercial laboratory for analysis (Figure 5-20).

Table 5-11. Characteristics of groundwater quality parameters determined pre- and post-deployment of the ISMA. Any difference observed were within the range expected in a well that is subject to tidal movement of groundwater.

	Pre-deployment	Post-deployment
Depth to water (ft)	3.3	3.6
Temperature (°C)	20.46	21.1
Dissolved Oxygen (mg/L)	9.92	5.96
ORP (mV)	83.1	240.7
pH	7.85	7.7
Conductivity (µS/cm)	1683	1148
Salinity	0.94	0.62

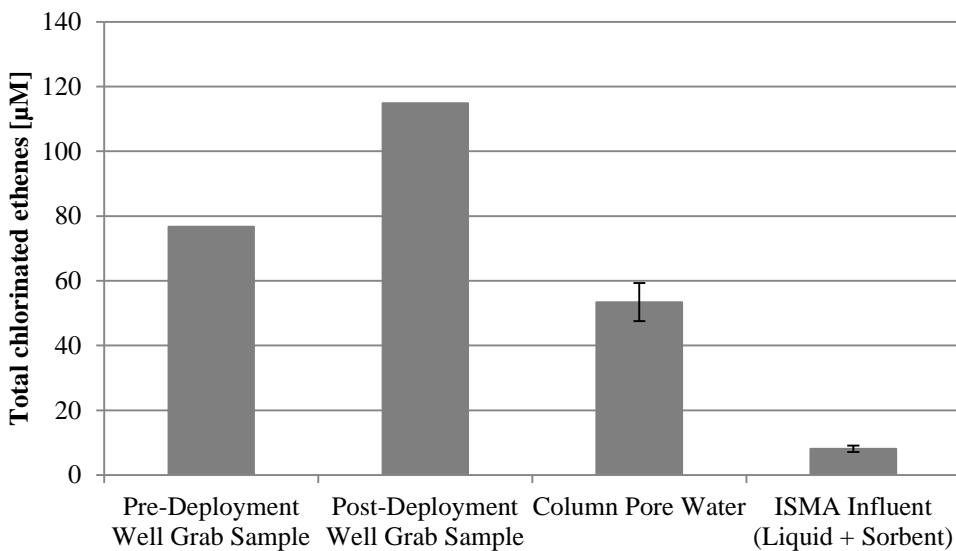


Figure 5-20. Sum of chlorinated ethenes (TCE, cDCE, and VC) detected in different sample types. Sum of chlorinated ethenes (TCE, cDCE, and VC) detected in the different samples types. Results indicate that columns were exposed to those concentrations of volatile organics found in

grab samples of the groundwater and that the lower concentrations observed in captured effluent are a result of losses due to extended effluent storage in the ISMA.

These results provide multiple lines of evidence for a successful conversion of aerobic site groundwater to anaerobic conditions that facilitated the reductive dehalogenation of TCE by the strict anaerobic bacteria (*Dehalococcoides*) added to the sediment. The reductive dechlorination of TCE in the presence of high concentrations of Cr(VI) (>5 mg/L) is a notable secondary outcome of this study. The observed biological removal of TCE in the presence of 24 mg/L of Cr(VI) in groundwater entering the ISMA extends the reported spectrum of conditions conducive to reductive dechlorination of chloroethenes via bioaugmentation.

While we can confidently conclude that we observed reductive dehalogenation in our biostimulation experiments, unfortunately, no such claims can be made about any attenuation processes that may have transpired in the MNA experiments. A 20% reduction of TCE mass was observed in MNA effluent, relative to the influent. However, the overall poor mass capture of volatiles in collected samples prevents one from drawing any definitive conclusions from this finding. Until better mass balance of volatiles in the effluent storage vessels can be achieved, the possibility cannot be ruled out that the observed 20% reduction in TCE mass simply was lost in the device via volatilization through the additional length of tubing and the column apparatus that the liquid had to traverse prior to collection in the effluent storage container. It should be noted, that we tried to minimize this loss by choosing compatible materials (Teflon and glass). Furthermore, any MNA processes that may have occurred in the sediment columns likely would have been relatively slow in comparison to losses observed in the bioaugmentation and biostimulation microcosms. To accurately quantify these processes one would need (i) a complete mass balance of TCE, or an alternative tracer compound to track attenuation, and (ii) a longer column residence time, and therefore a longer deployment time.

5.7.2 Industrial Site – ISMA Results

A flow-through field experiment was conducted with the ISMA testing MNA (experiment *A*) and bioaugmentation with a seed culture and sodium acetate (experiment *B*). In addition, an influent control was included in the experiment. Sodium acetate was chosen over ethyl lactate because in lab experiments, the addition sodium acetate resulted in higher numbers of perchlorate-reducing bacteria and did not lead to unwanted sulfate reduction.

Perchlorate was reduced over a period of 21 days from $228 \pm 1 \mu\text{g/L}$ to $30 \pm 37 \mu\text{g/L}$ by bioaugmentation (*B*), while perchlorate concentrations did not decline in MNA experiments (*A*), compared to the influent control (Figure 5-21). Sulfate was not reduced significantly in any of the samples (data not shown).

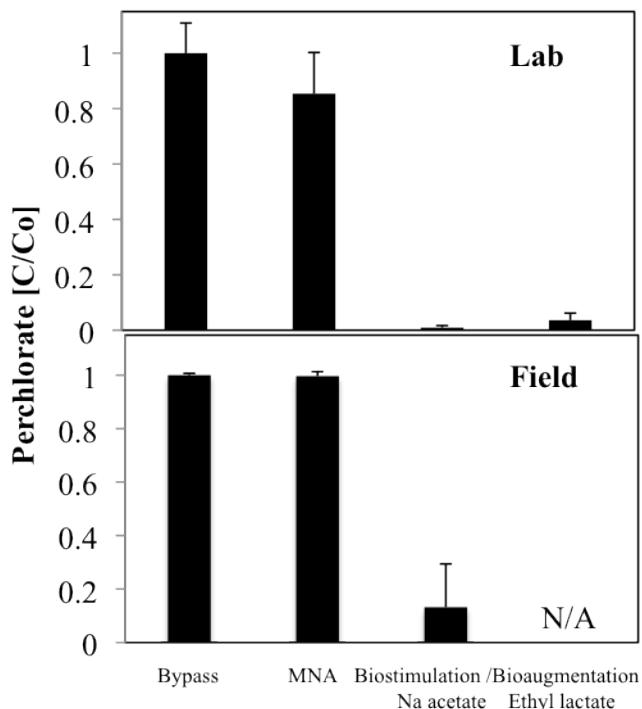


Figure 5-21. Concentration of perchlorate in different experimental groups normalized to influent (bypass). Data represent composite samples collected over 21 days, representing the whole duration of the experiments. N/A = not tested in field experiment. Error bars represent standard deviation.

By its design, the ISMA allows analysis of microbial communities in column effluent and sediment and to examine their spatial distribution across the columns. Sampling of both habitats has been recognized as essential to provide a complete picture of the microbial community

(Alfreider, Krossbacher et al. 1997; Lehman 2007). DNA analysis of effluent and sediment showed that perchlorate reducers mainly settled onto column sediment (concentration 2 - 3 orders of magnitude higher than in aqueous phase), while general bacteria were found at similar levels on sediment and suspended in the aqueous phase. Results further show that bioaugmentation with nutrient addition led to an increase in gene copy numbers of 16S rRNA (180-fold on average) and perchlorate reductase (*pcrA*) (690-fold on average), indicators for general bacteria and perchlorate-reducing bacteria, respectively (Figure 5-8). The column sediment was sectioned in three equal sections (inlet, middle, and outlet) and DNA copy numbers were analyzed. Results show that the majority of bacteria in all columns (lab and *in situ*) reside in the inlet portion of the sediment columns, which harbors 77 ± 20 % of general bacteria (Figure 5-22). This is even more pronounced for the columns that were bioaugmented, where around 90 ± 10 % of general bacteria were found in the inlet portion of the sediment columns. The reasons for this are likely two-fold: In bioaugmented columns nutrient concentration (carbon source and electron acceptors) at the inlet of the columns is highest, and therefore provides ideal growth conditions for bacteria leading to their high numbers. This has been found in several flow-through column studies (Bouwer and McCarty 1982; Hosein, Millette et al. 1997; Schäfer, Schäfer et al. 1998; Giblin, Herman et al. 2000). For MNA columns where no nutrients were added, different sediment filtration mechanisms (McDowellboyer, Hunt et al. 1986; Bolster, Mills et al. 2000) straining the bacteria from the incoming groundwater most likely caused the high DNA copy numbers found near the inlet.

While concentrations of general bacteria were similar between lab and field experiments, the concentration of perchlorate-reducing bacteria in the effluent and influent samples was about one order of magnitude lower *in situ* than in the lab experiment. Similar observations have been reported previously, where bacteria introduced through bioaugmentation were not able to compete with the indigenous community as effectively as predicted by lab studies (Mueller, Resnick et al. 1992; Zhang, Logan et al. 2005) or were subject to grazing by protozoa (Goldstein, Mallory et al. 1985; Ramadan, Eltayeb et al. 1990; England, Lee et al. 1993). These effects play a large role *in situ* and are one reason why *in situ* experiments are more valuable than similarly performed laboratory tests. At the same time, perchlorate-reducing bacteria were found in similar concentrations in the sediment for both lab and *in situ* experiments. This finding, which is in

contrast to the differences found in effluent concentrations, supports previous findings that bacteria attached to surfaces (sediment) or residing in small pore spaces are generally better protected from adverse environmental conditions and attack by grazers (Heijnen and Vanveen 1991; England, Lee et al. 1993; deLeo and Baveye 1997). Overall, the lower total number of perchlorate-reducing bacteria *in situ* is in accordance with the lower perchlorate reduction rate found in the field.

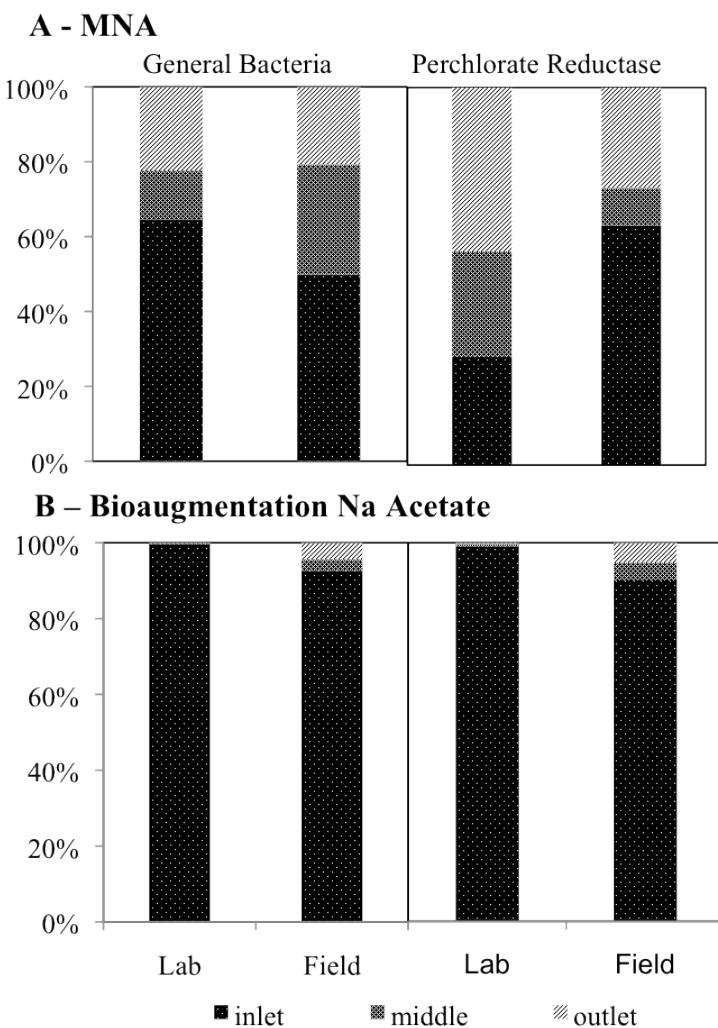


Figure 5-22. Relative bacteria distribution in sediment columns. Results of quantitative PCR targeting the 16S rRNA gene of general bacteria and perchlorate reductase (*pcrA*). Shown are relative results for all column sections.

6.0 PERFORMANCE ASSESSMENT

Table 6-1. Performance Objectives.

Performance Objective	Data Requirements	Success Criteria	Results
Qualitative			
Demonstrate capability of conducting mutually exclusive experiments in parallel in the same well	Monitoring of select water chemistry parameters	Evidence for mutually exclusive conditions in parallel experimental groups (i.e., aerobic, anaerobic).	Objective met
No residue released into monitoring well during testing	Water sampling and chemical analysis before and after ISMA deployment	Groundwater chemistry does not differ before and after ISMA deployment	Objective met
Determine potential side effects of remediation strategies	Monitoring data for potential adverse outcomes (e.g., heavy metal dissolution and leaching)	Mass balance for secondary contaminant (e.g., VC accumulation, Cr leaching) in various experiments reveal quantitative data for different simulated remediation approaches	Objective met
Quantitative			
ISMA study is cost-effective compared to a lab study of comparable scope	Compile cost data for ISMA and lab study	Cost of ISMA study is equal to or less than cost of lab study of comparable scope	Objective met
ISMA study is cost-effective compared to a field trial producing a similar data output	Compile cost data for ISMA and field trials	Cost of ISMA experiment is equal to or less than cost of field trial	Objective met
Reproduce outcome of prior lab studies in the ISMA	Monitoring of select water chemistry parameters	Available rates and trends determined in the lab can be reconciled with ISMA results. Rates between field and ISMA agree within an order of magnitude.	Objective met
Reproduce outcome of prior field trials in the ISMA	Monitoring of select water chemistry parameters	Available rates and trends determined in the field can be reconciled with ISMA results. Rates between field and ISMA agree within an order of magnitude.	Objective met

6.1 PERFORMANCE OBJECTIVE: Demonstrate capability of conducting mutually exclusive experiments in the same well

This objective was achieved at both deployment locations, namely the creation of aerobic and anaerobic conditions within parallel microcosms in the same well at the same time.

At NAS-NI, we conducted three mutually exclusive experiments, in the ISMA, in parallel in the same well. The natural attenuation columns were aerobic, reflecting the prevailing condition at the deployment location. We created anaerobic conditions in our bioaugmented and biostimulated columns. At NAS-NI, anaerobic conditions were evidenced by elevated levels of cDCE, VC, and ethene, which are understood to be only produced under reducing conditions.

At Site 2, two mutually exclusive experiments were conducted simultaneously in the same well. Experimental group 1 assessed natural attenuation (MNA) conditions where native, aerobic groundwater was pumped through sediment columns. No electron donor or microbial culture was amended. No significant changes in water chemistry (major anions, perchlorate, pH) were detected between MNA and control water that was not pumped through sediment columns (Figure 6-1), indicating that aerobic conditions were maintained in the MNA microcosms.

In Experimental group 2 we assessed bioaugmentation with a perchlorate reducing seed culture and sodium acetate as carbon source/electron donor. This lead to the generation of reducing/anaerobic conditions in the microcosms, as evidenced by observed reduction in nitrate and perchlorate concentrations (Figure 6-1). Microorganisms utilize nitrate or perchlorate as electron acceptor under anaerobic conditions, and reduce them to nitrogen gas or chloride and oxygen, respectively.

We have thus successfully demonstrated the ISMA's capability of creating mutually exclusive anaerobic and aerobic conditions in different sediment microcosms, but within the same ISMA deployment in an aerobic well. This is a scientific first and a major engineering achievement.

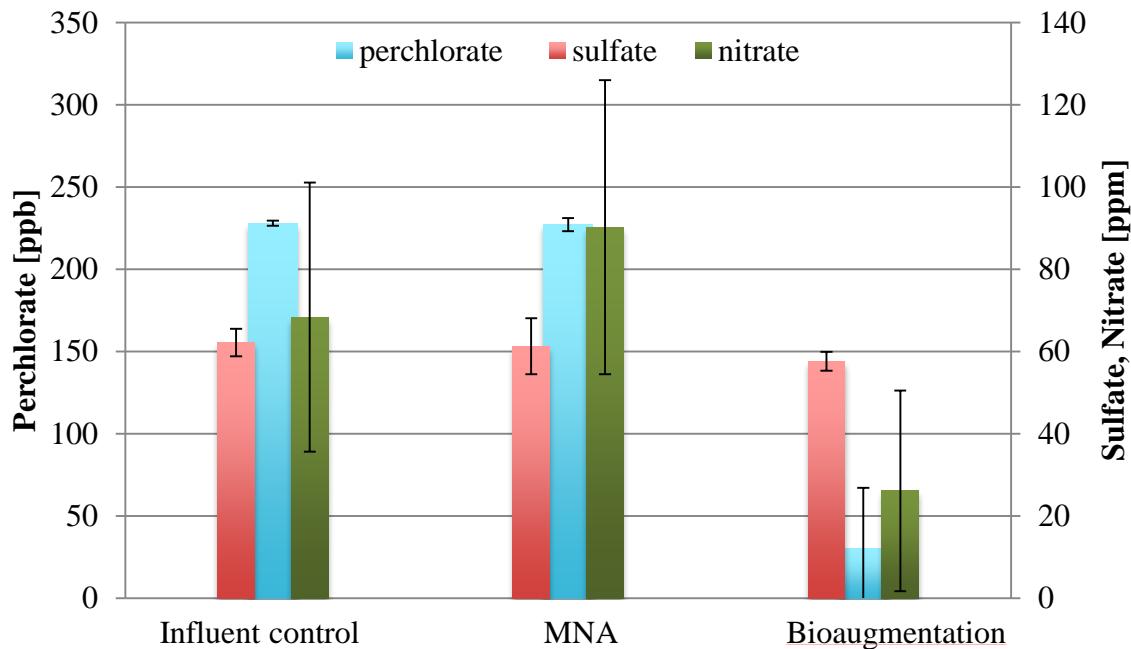


Figure 6-1. Anion concentrations in column effluent of different experimental groups. Values represent the average of triplicates; error bars represent one standard deviation. Nitrate concentrations are corrected by subtracting the nitrate contribution from the added preservative.

6.2 PERFORMANCE OBJECTIVE: No residue released into monitoring well during testing

This objective also was achieved. At both deployment locations, no negative water quality impacts were observed resulting from ISMA deployments. Pre- and post- deployment grab samples of the deployment well showed no appreciable differences that may have resulted from the ISMA deployment.

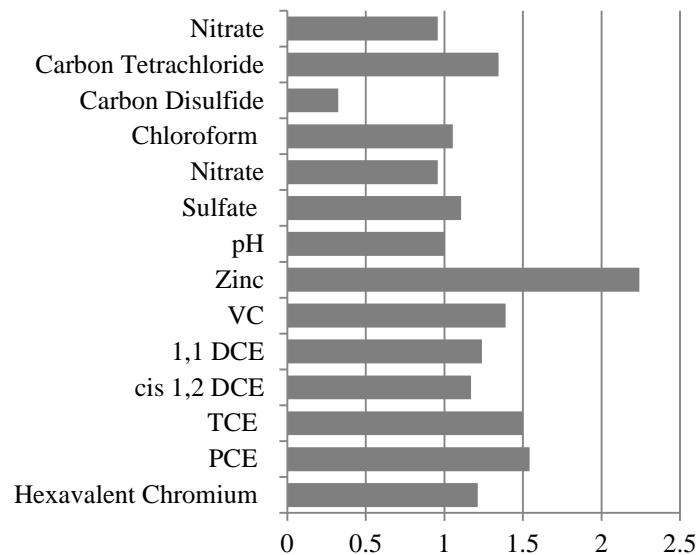


Figure 6-2. Groundwater chemistry in well grab samples before and after ISMA deployment at site 1. Change in Concentration after ISMA Deployment represented as C/C_0 . Fluctuations observed are due to tidal influence, additional parameters presented in Table 5-11.

The ISMA test at Site 2 was conducted in well MW-8. The well was sampled before and after deployment and analyzed for a range of parameters, including perchlorate, sulfate, nitrate, ammonium, and DNA concentration. Results for the ionic analytes shown in Figure 6-3 and the DNA concentration (26 and 18 ng/mL groundwater before and after deployment, respectively) showed no impact on groundwater quality by the deployment of the ISMA.

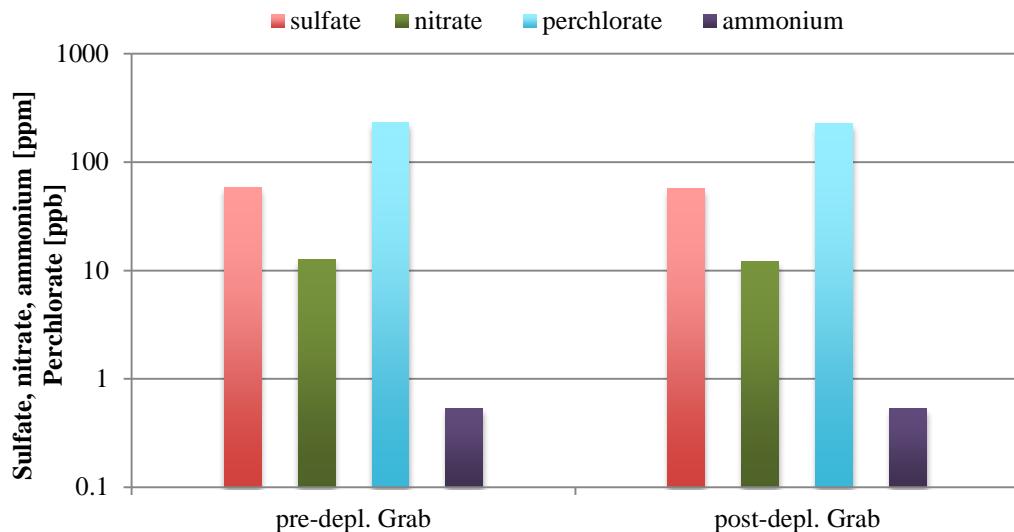


Figure 6-3. Groundwater chemistry in well grab samples before and after ISMA deployment at site 2.

6.3 PERFORMANCE OBJECTIVE: Determine potential side effects of remediation strategies

This objective also was achieved. We demonstrated the ISMA's ability to determine potential side effects of *in situ* remediation approaches under investigation and performed at a small scale in the device.

At NAS-NI, we successfully measured numerous parameters that potentially may have been secondary negative impacts of the remediation strategy, including pH and metals, where no negative impacts were found, as well as dechlorination byproducts, where elevated concentrations of VC were observed in the bioaugmentation experimental group. However, elevated concentrations of acetone (Figure 6-4) and 2-butanone (Figure 6-5) (also commonly referred to as methyl ethyl ketone) were detected in effluent from bioaugmentation and biostimulation experimental lines. These are fermentation products that have in the past been detected transiently immediately after biostimulation was implemented (Fowler, Thompson et al. 2011). They may also be laboratory artifacts arising from analyzing samples with high VFA content. The fact that this result was noted in the ISMA and not in batch bottles suggests the ISMA is a capable tool for determining unexpected potential side effects of *in situ* remediation.

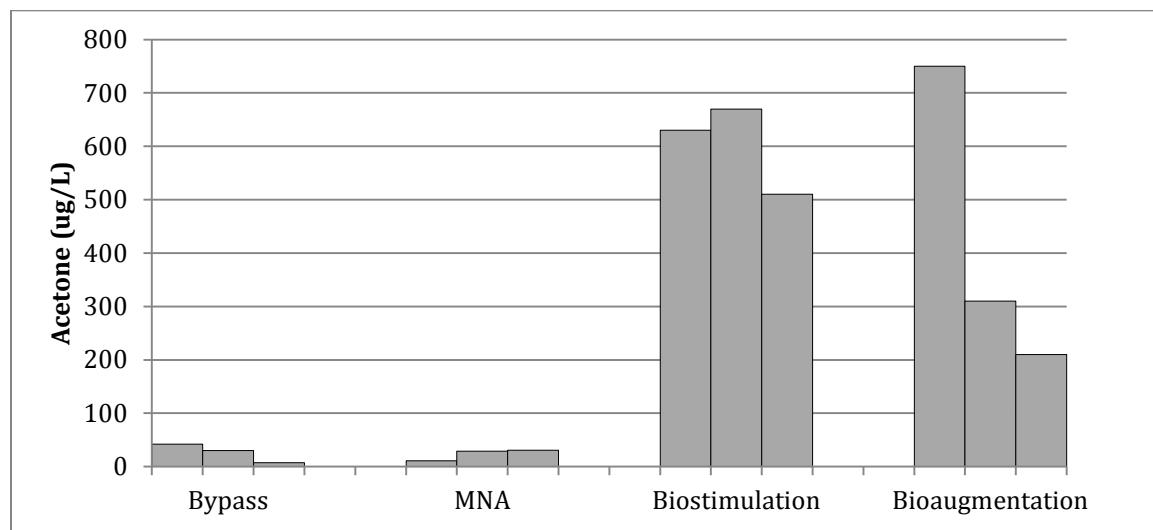


Figure 6-4. Acetone concentrations detected in ISMA effluent after incubation *in situ* at NAS-NI.

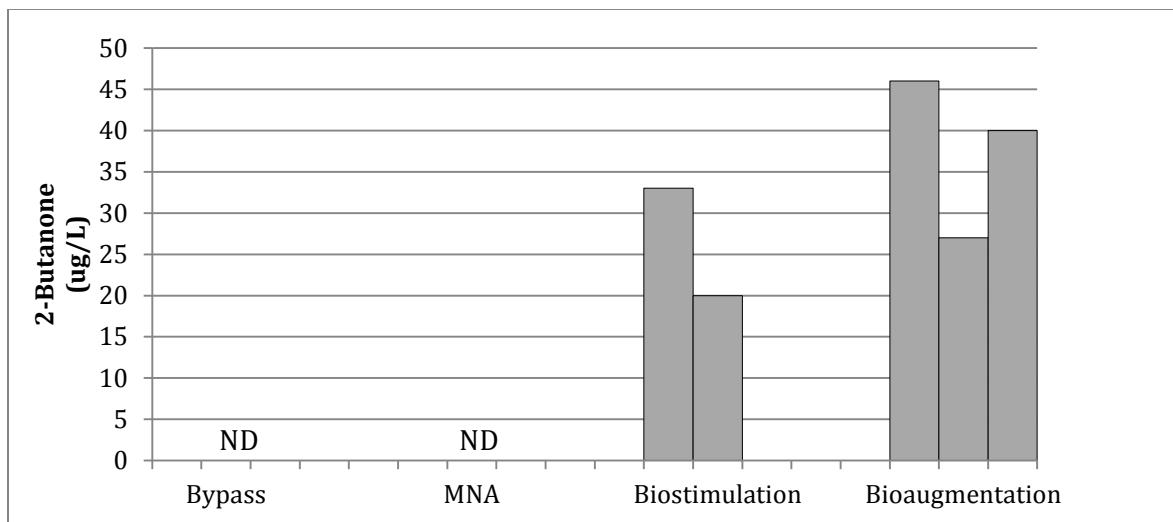


Figure 6-5. 2-Butanone concentrations detected in ISMA effluent after incubation *in situ* at NAS-NI.

At Site 2, nitrate concentrations in groundwater were found around 10 ppm, which is below the maximum contaminant level (MCL) of 45 ppm set by the U.S. Environmental Protection Agency. Under anaerobic conditions, this nitrate can be reduced to nitrite by denitrifying bacteria, and therefore lead to concentrations above the MCL for nitrite of 5 ppm. We therefore monitored for concentrations of nitrite in column effluents. Results showed that nitrite (2 ppm) was only detected in effluent from one bioaugmentation column, which was below the MCL.

Another side effect commonly observed with the addition of organic carbon sources to the subsurface is lowering of the pH through production of carbonic acid. Consequently, pH was determined in all column effluents and well grab samples. No significant changes on the pH (7.7 – 8.7) were detected in any of the samples.

6.4 PERFORMANCE OBJECTIVES: ISMA study is cost-effective compared to a lab study of comparable scope

This objective also was met. The ISMA was found to be cost effective to a comparable lab study. Please refer to section 7.0, COST ASSESSMENT, for a detailed analysis.

6.5 PERFORMANCE OBJECTIVES: Compare cost of conducting ISMA study vs. field trial

This objective also was met. The ISMA was found to be cost effective compared to a field pilot trial. Please refer to section 7.0 for a detailed analysis. Please note, a comparison was possible only for the NAS NI Deployment (Site 1), as no comparable field pilot trial was conducted at Site 2.

6.6 PERFORMANCE OBJECTIVE: Reproduce outcome of prior lab studies in the ISMA

This objective also was met. ISMA results from the two deployments are presented.

6.6.1 NAS North Island

No rates were calculated in the neither the report from the bench-scale treatability study nor the field scale pilot study conducted by third parties at NAS-NI, so we calculated rates where applicable to generate a basis on which to compare treatability study methods. Table 6-2 and Table 6-3 report the various first-order rate constants calculated.

Table 6-2. TCE: calculated first-order degradation constants ($k \text{ day}^{-1}$).

Amendments	Lab Batch Bottle	Field Pilot Trial	ISMA
Lactate	0.051 ± 0.043	-	-0.001 ± 0.157
Lactate + KB-1®	-	-	0.481 ± 0.048
SRS + nutrients + KB-1®	0.524 ± 0.002	-	-
SRS-M + nutrients + KB-1®	3.358 ± 0.169 (in mineral medium)	0.240 (maximum rate detected)	-

Table 6-3. Cr(VI): calculated first-order degradation constants ($k \text{ day}^{-1}$).

Amendments	Lab Batch Bottle	Field Pilot Trial	ISMA
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Lactate	0.086 ± 0	-	0.385 ± 0.104
Lactate + KB-1®	-	-	0.479 ± 0.113
SRS + nutrients	0.117 ± 0	-	-
SRS-M + nutrients + KB-1® (in mineral medium)	7.948 ± 1.218	0.247 (maximum rate detected)	-

Laboratory Batch Bottle rate constants were calculated as follows:

$$k = \frac{\ln(C_{baseline}) - \ln(C_{treatment})}{t_{treatment} - t_{baseline}}$$

where $C_{baseline}$ and $t_{baseline}$ were the concentration and time point prior to amendment, respectively, and $C_{treatment}$ and $t_{treatment}$ were the contaminant concentration and time point when the contaminant was no longer detectable, or at the last sampling point, whichever was sooner.

The rate was calculated in this manner for each replicate bottle. The average and standard deviation of the rate constants is reported in Table 6-2 and Table 6-3. Note, for the SRS + nutrients + KB-1®, $C_{baseline}$ and $t_{baseline}$ for the TCE rate constant was taken from the last sampling point prior to KB-1® amendment; the associated graph is shown in Figure 6-4.

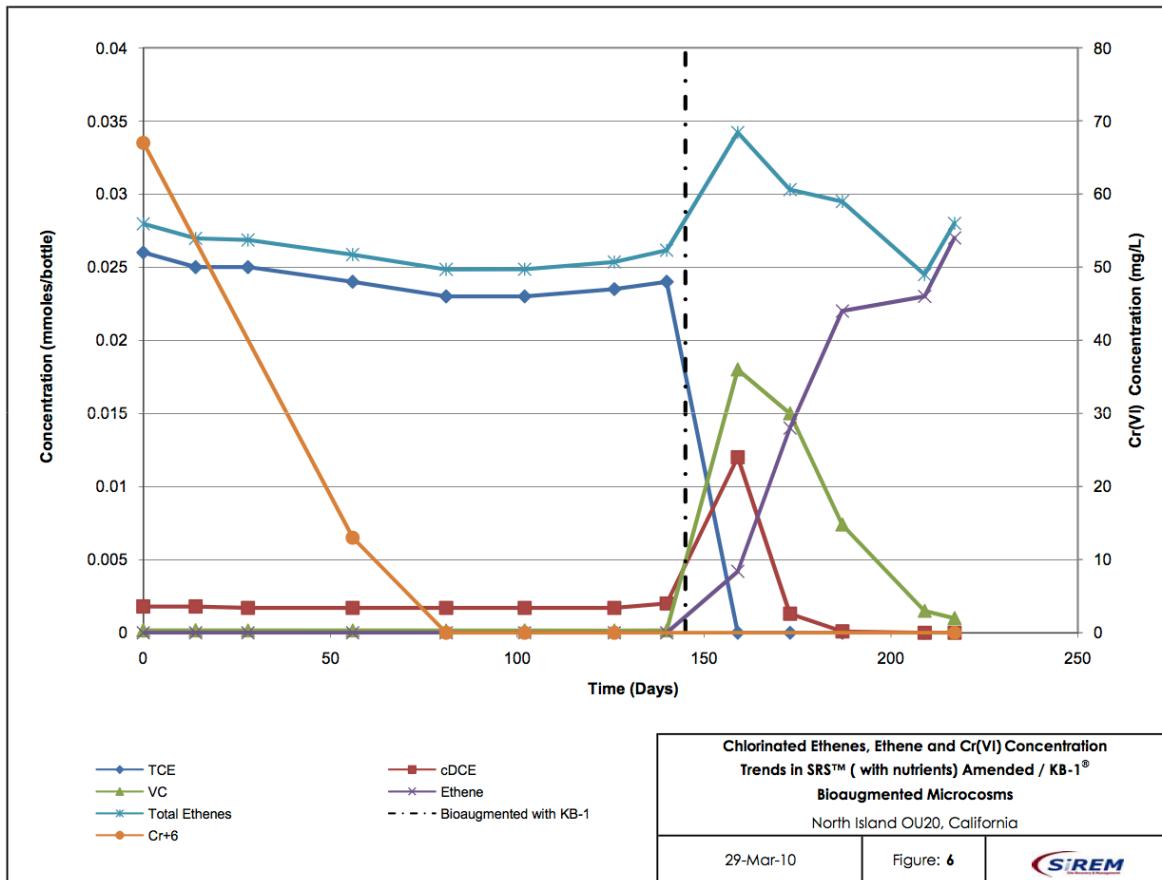


Figure 6-6. Sampling results from a batch bottle microcosm study performed by SiRem, demonstrating the effectiveness of bioaugmentation with KB-1® for treatment of groundwater from OU-20 at NAS-NI.

Rate constant values generated from an ISMA deployment were calculated as follows:

$$k_{Composite} = \frac{\ln(C_{iInfluent}) - \ln(C_{iEffluent})}{\Delta T_{Column}}$$

where,

ΔT_{Column} = residence time within the column

$C_{iInfluent}$ = the average concentration in the MNA experimental group effluent

$C_{iEffluent}$ = the concentration of the contaminant in the column effluent collected throughout the *in situ* incubation period.

This is similar to the approach described in section 5.6, with the additional correction that concentrations detected in the influent and effluent are composite samples. The MNA

experimental group is taken as the influent baseline due to the fact that an incomplete mass balance might be the result of volatilization losses through the column assembly, and attributing those losses to biodegradation would yield an overly optimistic rate constant.

Field pilot trial results were inconsistent. A few of the monitoring wells showed relatively rapid reduction, but some showed no appreciable differences, or rapid rebound after SRS-M injections. Consequently, only the maximum rate constant calculable from a single monitoring well is reported. Variable sourcing for rate calculates are summarized in Table 6-4:

Table 6-4. Variable Sourcing for Rate Calculations at NAS-NI.

Variable	Lab Batch Bottle	Field Pilot Trial	ISMA
$C_{control}$	Baseline concentration at T_0	Baseline concentration at T_0	Concentration in bypass (influent concentration)
$C_{treatment}$	Concentration after treatment and after no further activity was observed	Lowest concentration detected in treatment well	Concentration in treated effluent
ΔT	Time between $C_{control}$ and $C_{treatment}$	Time between $C_{control}$ and $C_{treatment}$	Calculated column residence time

6.6.2 Industrial Site - Degradation Rate Calculation

For laboratory treatability studies, contaminant concentrations are monitored over time and degradation rates are determined from time-discrete data (equation 1 and 2). Natural log transformed concentration data were plotted against time. We fitted all data sets with a linear regression revealing a first-order degradation rate of $0.05 \pm 0.02 \text{ hr}^{-1}$ with a correlation coefficient R^2 between 0.62 – 0.97 for five replicates. Overall, using a first-order approximation of Monod kinetics provided a reasonable fit for the perchlorate concentration profiles of the experiments conducted in this study.

In the configuration used for the field column study, the ISMA allowed collection of only a single composite sample per column, which was used to estimate the degradation rate from

triplicate measurements by employing equations 3 and 4. To determine the magnitude of the impact caused by determining rates by this composite approach, we used the lab flow-through experiment to calculate degradation rates from both time-discrete and composite sampling. On a conceptual level, composite samples will yield inherently conservative rates, since they represent an average of the adaptation phase (when the contaminant is not reduced) and steady state (stable contaminant degradation). The extent of underestimation of the “true” degradation rate depends on the relative duration of adaptation vs. steady state.

In the lab flow-through experiment, the time-discrete rates were on average 46% ($\pm 21\%$) higher than those calculated with composite concentrations (Table 6-5). The lag phase before steady-state contaminant reduction was only two days (or 10%) of the total duration of the experiment (Figure 5-6). We therefore expect the composite degradation rate determined *in situ* to underestimate the “true” rate by approximately 46%. Further refinement of estimates may be achieved by implementing a switching valve in the ISMA to fractionate the volume of effluent and thereby obtain time-resolved data.

Table 6-5. Overview of first-order reduction rates for Site 2. Results from lab and field experiments are listed.

Conditions of Lab or Field Experiments	Composite Reduction Rate [hr-1]	Time-discrete Reduction Rate [hr-1]
<i>Laboratory – Batch:</i>		
Biostim Ethyl Lactate	n/a	0.05 ± 0.02
<i>Laboratory – Flow-through:</i>		
Natural Attenuation	0.02 ± 0.01	n/a
Bioaug Sodium Acetate	0.55 ± 0.21	0.84 ± 0
Bioaug Ethyl Lactate	0.35 ± 0.06	0.84 ± 0
<i>Field – Flow-through:</i>		
Natural Attenuation	< 0.003	n/a

Bioaug Sodium Acetate	0.24 ± 0.15	n/a
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n/a = data not available

The perchlorate reduction rate (composite rate) determined *in situ* was half of that determined in the lab flow-through experiment for bioaugmentation with sodium acetate. The performance objective has therefore been met (degradation rates within same order of magnitude).

6.7 PERFORMANCE OBJECTIVE: Reproduce outcome of prior field trials in the ISMA

Please refer to section 6.6 for the relevant discussions regarding the demonstration at NAS-NI. Please note, a comparison was possible only for the NAS NI Deployment (Site 1), as no comparable field pilot trial was conducted at Site 2.

7.0 COST ASSESSMENT

The following section details some of the costs associated with ISMA field deployments. Where applicable, costs are provided for the ISMA demonstrations detailed, but the focus in the cost assessment is to determine projected costs for future ISMA deployments, and to compare them to alternative methods of conducting treatability studies.

7.1 COST MODEL

Cost model has been broken down into three broad categories: materials, sample analysis, and labor.

Table 7-1. Direct material costs incurred during NAS-NI deployment.

Cost Element	Unit Cost	NAS-NI QTY	Total cost NAS-NI deployment
ISMA consumables			
Viton tubing 0.89 mm ID	\$105 / 50 ft	100 ft	\$210
Viton tubing 3.17 mm ID	\$44 / 25 ft	12 ft	\$22
Effluent containers	\$40 / piece	12	\$480
GAC cartridges	\$68 / 50 tubes	12	\$17
Subtotal			\$729
Field equipment			
Cable Ramps	\$68/3ft/month	60ft for 1 month	\$1,360
YSI meter	450 / week	2 weeks	\$900
Boom truck + operator	\$100/hr	6	\$760
Subtotal			\$3,020
Total			\$3,749

ISMA consumables are non-reusable components of the ISMA. The bulk of the ISMA device - including the columns, pumps, motors, internal skeleton, outer shell, and electrical connectors - is reusable. However, to minimize the risk of cross-contamination between deployments, internal components that come into contact with field materials are replaced. These materials are: flexible tubing (used to route groundwater throughout the device), peristaltic pump tubing, tubing connectors and fittings, check valves, syringes for amendment injection, effluent storage containers, and activated carbon sorbent cartridges for capture of volatile organics (if applicable).

Field equipment includes the costs associated with storage of ISMA equipment on site. Necessarily these are site-specific costs. For a deployment location that is secure and sparsely used (our deployment at the industrial site), no such costs exist. At NAS-NI, where the deployment location was in an active parking lot, cable ramps and a custom well box cover were necessary to avoid any impact on normal traffic flow. Examples of costs that might fall into this category at other locations may include installing a temporary shed or a fence to protect ISMA equipment.

Additional field deployment cost is the rental of a boom-truck and operator. In the current implementation of the ISMA, this is necessary. However, the ISMA is compact enough that in the future, deployment may be feasible with only a tri-pod or custom hoist, thereby eliminating the additional expenditure linked to boom truck operation.

Table 7-2. Direct costs for sample analyses by commercial laboratory incurred during NAS-NI deployment.

Sample Analysis - Method	\$ / Sample	NAS-NI QTY	Total cost NAS-NI deployment
VOC - 8260B	100	14	\$1,400.00
CAM (17) Metals - 6010/7000	150	14	\$2,100.00
Anions (3 anions) - 300.0	45	14	\$630.00
pH - 9040	15	14	\$210.00
TDS - 160.1	15	14	\$210.00
VFA - 300 Modified	100	14	\$1,400.00
Hexavalent Chromium - 7196	60	14	\$840.00
Subtotal			\$6,790.00

Laboratory Analysis: This category consists of chemical analysis of samples generated during the course of an ISMA deployment. This can be performed by a certified commercial laboratory, by the site owner, or at the research laboratory at Arizona State University. Sample analysis costs will likely differ between deployments based on data needs and relationships with commercial labs.

Table 7-3. Projected labor needs for future deployments.

Deployment Activities	Project Manager	Senior Technical Advisor	Environmental Scientist / Engineer	ISMA Technician
Prepare ISMA Configuration for Deployment (includes mechanical build, systems check, column conditioning)	4 wk @ 10%	4 wk @ 10%	4 wk @ 20%	4 wk @ 100% FTE
Pack/Ship ISMA to Customer Site	1 wk @ 10%			1 wk @ 80% FTE
Receive/Secure ISMA at Customer Site	1 wk @ 10%			
Deploy ISMA Down-Hole and Initiate Process Run (includes travel time)	1 wk @ 100%			1 wk @ 100%
Stop Process Run; Retrieve ISMA Samples and deliver to commercial laboratory for analysis	1 wk @ 100%			1 wk @ 100%
Data reduction and analysis; Reporting	4 wk @ 25%	4 wk @ 50%	4 wk @ 50%	4 wk @ 55%
Subtotal (Person-Months)	0.975	0.6	0.7	2.3

Direct labor costs incurred during the demonstration deployments are not reported or computed, partly due to the difficulty of quantifying the exact effort expended on any single-deployment, and of differentiating from efforts for ISMA development and iterative design, and from concurrent associated laboratory studies, but also because such a computation would not be instructive of future costs. With two ISMAs built, and over 10 individual deployments that were used to iteratively improve on the ISMA and identify and correct failure modes, the one-time capital and labor costs have been incurred, and future deployments will be significantly less expensive. We have identified and listed in the next few paragraphs the effort that will be required in future deployments and listed in Table 7-3 the projected personnel time required.

Laboratory Labor: Column microcosm assembly and preparation consists of sediment processing (drying, homogenizing, crushing and sifting as necessary) and then manually packing the columns with processed sediment. ISMA assembly consists of replacing and installing all the consumable materials, testing all channels for consistent flow rates and leaks, and loading the materials and reagents necessary for the test (*in situ* treatment technology, preservative, and sediment columns, etc.).

Column operation and preconditioning in the laboratory is not included in the labor model of an ISMA deployment due to the fact that it can and should be considered as a stand-alone laboratory column treatability study. It is complementary, but not strictly necessary, to an ISMA deployment.

Field Labor: A boom truck and operator are necessary for approximately 2-3 hours during both ISMA deployment and retrieval. Additional support is required is by one ISMA engineer. During deployment, this consists of taking a well grab sample before and after deployment, and determining field parameters with a pre-calibrated multi-parameter probe. The ISMA engineer together with the boom truck operator installs the ISMA in the well, and retrieves it after field incubation. The ISMA engineer ensures that all electronic components (solar panels, battery array, controls for ISMA pumps and motor) function properly. ISMA equipment is stowed on site in such a way that it minimizes impact on site activities and minimizes risk of vandalism or theft. During retrieval, additional tasks include external decontamination of the ISMA, sample extraction from the ISMA, transfer of samples to the containers and currier designated by the commercial lab performing analyses.

7.2 COST DRIVERS

There are relatively few site-specific cost drivers that may drive up the cost of an ISMA deployment. Beyond column preparation, and the chosen amendment and quencher, ISMA assembly and preparation is not specifically sensitive to cost variation based on deployment site. The largest site specific cost driver is the type and number of sample analyses that are dependent on the data needs of the customer, which may also include the need for additional ISMA deployments or laboratory studies.

An additional cost driver not incurred during the demonstration deployments but recommended for future deployments is the cost of collection of fresh sediment for microcosm construction. This cost of drilling a well and collecting the sediment is highly site-specific, and therefore not enumerated in our cost analysis.

One of the largest overall cost drivers for a treatability study that incorporates the ISMA will hinge on the decision of whether to conduct a complementary laboratory study. A laboratory column study prior to field deployment will yield empirically generated column operation parameters. Data generated from such a laboratory study can maximize the utility of a field deployment by informing the field experimental design on dosing requirements, column residence times, and other design parameters. A complementary laboratory column study may also be particularly beneficial if the *in situ* treatment technology being evaluated is dependent on a slow-growing microbial culture that may require an extended acclimation period in the column before demonstrating significant activity.

7.3 COST ANALYSIS

Table 7-4 lists the calculated projected costs for ISMA deployments in the immediate future, and potential deployment costs, once certain process optimizations and economies of scale are realized. As mentioned in the previous subsection, there are relatively few site-specific cost drivers, thus the costs listed are representative of those that might be incurred during a typical deployment. Assumptions underlying this claim are that the ISMA study site is similar to the demonstration locations, namely that

- A single deployment may satisfy the initial data needs
- There is a pre-existing 4"-ID monitoring well that can accommodate the ISMA
- ISMA surface components can be accommodated safely for the deployment period

Table 7-4: Projected ISMA costs.

Cost element	Present	Future
Labor costs	\$41,515	\$20,757
Consumable and Equipment Costs (not including ISMA leasing)	\$7,989	\$1000
Laboratory analysis	\$14,000	\$12,000
Travel	\$4,000	\$3,000
Facility and Administrative costs	\$43,210	\$29,924
Subtotal	\$110,713	\$66,681

Projected future cost reductions can be attributed to:

1. Labor reduction: economies of scale and efficiency will result from having multiple ISMA deployments ongoing concurrently, i.e., it does not take twice as much effort to assemble two ISMAs as opposed to one. The reduced labor costs presented are estimates are based a labor model which assumes three ongoing ISMA deployments at any one time. Similar economies of scale are already realized by other contract laboratories to which the ISMA technology is compared here.
2. Consumables and Equipment Cost: additional engineering effort can lead to refinements and reduced material needs per ISMA deployment. These modifications can be based on a redesigned, and reusable, effluent storage array, as well as hard-wired and easily serviceable liquid flow channels in the ISMA.
3. Laboratory analysis: the modest savings listed are primarily due to a customer-loyalty program and reduced unit cost when ordering a large number of analyses. This number will fluctuate based on customer needs, and is only included as an estimate assuming a standard suite of analyses chemical and microbial analyses for 14 samples (12 ISMA effluent channels, and deployment well samples before and after deployment).
4. Savings: With further modifications to the ISMA, it will be feasible to deploy with only two ISMA people on site, as opposed to the three that were present during the technology demonstration.
5. Facility and Administrative (F&A): These are a fixed percentage cost based on modified total direct costs. These are based on the costs at Biodesign-ASU, but are comparable to the overhead charges incurred in other academic or commercial settings.

It is instructive to compare ISMA costs to those for a standard laboratory treatability study of field pilot scale. In that regard, we collected the costs incurred at NAS-NI for the laboratory treatability study and field pilot scale, presented in Table 7-5.

Table 7-5. Feasibility study project costs: OU-20, NAS-NI.

Cost Element	\$
Project Management	\$71,435
Plans	\$88,633
Installation of Wells and Associated Sampling	\$80,527
Bench-scale Treatability Evaluation	\$53,424
Field-scale (Pilot) Treatability Evaluation	\$223,731
Reporting	\$94,883
Total	\$612,633

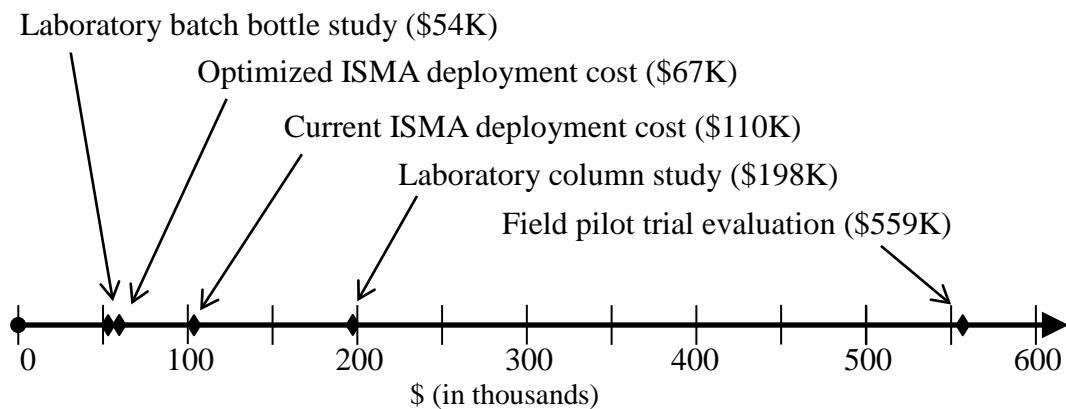


Figure 7-1. Cost comparison of treatability study methods.

Comparing costs incurred at NAS-NI, to projected costs for a comparable ISMA deployment, we see that an ISMA deployment is more expensive than a laboratory batch bottle treatability study but significantly less so than a field pilot trial. This is expected due to the fact that the ISMA produces results that are more representative of the field than a laboratory study, but generates them with significantly less impact than a field pilot trial.

It is also instructive to compare ISMA costs to those of a traditional column study; one commercial laboratory quoted a column study examining bioaugmentation at \$22,000 / column. At this rate, a lab study comparable to the ISMA, meaning, with 9 columns, the number of columns in the ISMA, brings the total cost to \$198,000. On a true comparison of flow-through to flow-through treatability study, the use of the ISMA can realize significant cost savings.

Furthermore, due to the standardized ISMA components, the marginal cost of additional columns in study will be significantly less than the fixed cost of \$22K / column, and this cost-savings realized by the ISMA will significantly increase with increasing complexity of the proposed study.

With respect to the performance objective of cost-efficiency, we conclude that it was met. The field demonstration produced data from flow-through experiments conducted *in situ*. Conducting a similar study with conventional columns in the laboratory is a cost-prohibitive endeavor that has not been attempted yet. Indeed, the miniaturization of column studies and the modular design of the ISMA gear make it feasible now for the first time to run many more than three flow-through sediment columns at a time for one and the same site. Whereas no competing strategy exists to obtain the kind of data furnished here, the ISMA technology enabled column studies that were in the range of costs typical of much simpler batch studies, as shown by the data in Figure 7.1. Thus, an additional not specifically anticipated outcome of the present work is that it produced a working model for how to conduct many flow-through sediment studies cost-effectively in the laboratory as well as in field.

8.0 IMPLEMENTATION ISSUES

The ISMA, while conceptually simple, is mechanically complex. With multiple modular components and independent lines, there exist significant opportunities for ISMA failure in the field due improper assembly from user error in the laboratory. To systematically identify, isolate, and minimize the opportunities for these errors, we have performed a Failure Mode Effects Analysis (FMEA), and installed safeguards in place to minimize failure modes in the future.

As part of these efforts, we have developed a comprehensive checklist (Appendix C) that ISMA technicians can use in the future to minimize user-error from minor oversight. Additionally, at the conclusion of this project, there will exist a video training resource that demonstrates all the steps involved with the laboratory assembly and field deployment of the ISMA.

Further development of the ISMA may be desired to broaden its applicability. Two high-priority potential ISMA improvements identified in this work are to develop a capability for real-time sensing and to improve the efficiency of capture of volatile organic compounds in the ISMA.

There are no regulatory barriers we have identified that may impede ISMA field deployment. In light of the fact that the ISMA does not release any compounds into the deployment well, it falls in the same regulatory category as field sampling devices. As such, an ISMA field deployment should not require additional permitting or approval beyond that necessary for field use of groundwater sampling devices. However, additional permitting may be necessary for storage of ISMA surface components.

ISMA use in remedial design will have to be subject to approval by site-relevant regulatory agencies. However, in light of the quality and field-relevance of data output, we anticipate no additional difficulties in securing this approval compared to a standard bench-scale treatability study.

Our team has engaged potential industrial partners and technology users; further laboratory and field deployments of the ISMA technology will prove helpful in identifying technology-specific benefits and challenges.

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10.0 APPENDICES

10.1 Appendix A: Points of Contact.

POINT OF CONTACT Name	ORGANIZATION Name Address	Phone Fax E-mail	Role in Project
Rolf Halden	The Biodesign Institute at Arizona State University; Center for Environmental Security; 781 E. Terrace Road Tempe, AZ 85287-5904	480-727-0893 halden@asu.edu	Principal Investigator
Kristin McClellan		480-727-0698 Kristin.McClellan@asu.edu	Project Manager / Researcher
Tomasz Kalinowski		480-727-0698 Tomasz.Kalinowski@asu.edu	Researcher

10.2 Appendix B: Synthetic groundwater recipe

The media for column study was prepared as detailed in “Enrichment, Cultivation, and Detection of Reductively Dechlorinating Bacteria” by Löffler FE, Sanford RA and Ritalahti KM, published in *Methods in Enzymology*, 2005 (397; 77- 111), but with the following modifications:

- Media was prepared under aerobic conditions, and no effort was made to create anaerobic conditions
- No rezazurin was added
- All reducing agents were omitted, namely L-cystine and sodium disulfide
- AATC vitamins supplements were reduced to 10% of the recommended dosing
- Carbonate was omitted due to the inability of maintaining a desirable pH in media at equilibrium with air. Buffering capacity was replaced with a 10mM phosphate buffer (to make 10L, amend 12.8g of dibasic potassium phosphate and 1.65g of monobasic potassium phosphate to get a target pH of 7.3).

10.3 Appendix C: ISMA checklists

Table 10-1. Packing list for ISMA field deployments

FROM LAB:	FROM STORAGE:
sample labels	windex wipes
cooler	sand bags
sample bags	ISMA deployment cap
sample bottles	2 security well-head clamps
freezer cold packs	clamp + wrench for assembly
nutrients	inverter
syringes + needles	3way plug
nitrile gloves	charge protector
silicone lubricant	battery cables + wingnuts
trash bags	plastic sheet for batteries
label tape + markers	gas vent line
note pad	
luer fittings + caps	rodent insulation
zip ties	work gloves
zip tie gun	water level meter
screws for ISMA connectors	bailer
o-rings for ISMA connectors	bailing string
duct tape	safety straps
electrical tape	hard hats
allen wrench for:	power cable for NIU
connector units	25ft steel cables
pump cassettes	2ft steel cables (short sections)
cassette set screws	safety vests
easy drain screw	cross bar for suspension over well head
paper towels	shackles
DI water for rinsing	orange safety vests
Liquinox detergent	traffic cones / delineators
rags for decon	
sample container for field parameter measurement	
extra connectors:	
luer male + female	tool box including:
10-line multi-connect	adjustable wrench for cable clamps
single quick connect	needle-nose + diagonal pliers
ISMA module connectors A + B	shipping permit/paperwork
laptop for NIU	ISMA sections 1-3
balance to weigh effluent bags	control unit box
	14 batteries
	electrical cables for unit
	3 solar panels
	solar panel frames + wingnuts
	cinder blocks
	deck box
	canopy tent

Table 10-2. Failure modes and effects analysis for ISMA

<i>Component and function</i>	<i>Failure Mode</i>	<i>Effect(s) of Failure</i>	<i>Potential cause of Failure</i>	<i>Current Controls, Prevention</i>	<i>Current Controls, Detection</i>	<i>Recommended Action</i>	<i>Action taken</i>
SS unit interconnects	Corrosion	Material degradation	Exposure to water in well	Corrosion resistant stainless steel	None	Better grade stainless steel; coating with corrosion resistant film	All exposed SS parts were chromium plated
SS unit interconnects	Leak	Water intrusion into unit	Neglected to install O-ring	None	None	Install O-rings pre-deployment	
Bulkhead fittings	Leak	Water intrusion into unit	Plastic fittings not strong enough	None	None	Replace with metal fittings	
Viton intake tubing (large)	Blockage	No flow	Tubing kinked	None	None	Reinforce tubing wall	Spiral wrap around tubing to prevent kinking
						Use thicker wall tubing	Smaller diameter with same wall thickness used (1/8" ID instead 1/4"ID)
Viton individual tubing (small)	Blockage	No flow	Kinking on top of columns from electrical connectors pushing down	None	None	Protect top of columns from electrical connectors	Separate compartment for electrical connectors
						Shorten electrical connectors	Shorten shrink wrap seal around connectors, shorten length of electrical connectors
Viton individual tubing (small)	Blockage	No flow	Pinched tubing in several places	None	None	Cut tubing to exact length to avoid excess loops	Tubing length adjusted
Viton individual tubing (small)	Blockage	No flow	Column sediment lodged in tubing	Pre- and post-column filters	None	Find more effective filters	
Groundwater intake	Leak	Water intrusion into unit	Twisting of viton tubing lead to tear	None	None	Use freely rotating swivel	Swivel installed

SS shell	Dent	Disassembly of unit very difficult (parts got stuck)	Damage during transport	Simple wooden frame for transport	None	Redesign intake so only one line comes out	Intake line combined to one outlet
HgCl2 preservative in effluent bag	Leak	Exposure of field personnel during handling	Loose connection on top of bag	None	None	Secure all units during transport; prevent other things from dropping onto it	Ratchet straps used to keep equipment in place
HgCl2 preservative in effluent bag	Growth of bacteria	Biological degradation of effluent post sediment column	HgCl2 resistance	None	Monitor for bacterial growth	Choose preservative that is non-toxic to humans	New preservative Kathon
Effluent bag	Growth of bacteria	Biological degradation of effluent post sediment column	Insufficient mixing within bag	None	Monitor for bacterial growth	Choose preservative that has wide range of effect	New preservative Kathon
Effluent bag frame	Effluent bags not held taught	Inefficient mixing within bag	Frame dimensions miscalculated			Install tube at bag inlet that delivers sample to preservative in the bottom of the bag	Frame elongated, spring incorporated to maintain tautness of bag.
Effluent bag connections	Insufficient working room for tubing connections in effluent bag frame	Troubleshooting, ISMA assembly unnecessarily difficult	Frame not originally designed to accommodate vent tubing.			Redesign frame	Effluent bag mounts on frame redesigned and simplified, cutouts for tubing enlarged.
Grommets on effluent bags	Corrosion	Mainly cosmetic	Contact of 2 different metals and water	None	Visual inspection	Use corrosion resistant material or prevent metal-to-metal contact	Stainless steel grommets
Peristaltic pump section	Pinched tubing	No flow	Too little space for tubing to bypass cassettes holding pump tubing	None	None	Redesign geometry of cassettes to provide more space	Cassettes combined on 2/3 of the radius, leaving 1/3 to bypass tubing

Peristaltic pump cartridge	Insufficient flow, tubing destroyed	No flow	Cartridge not correctly designed to accommodate tubing flex	None	Redesign cartridge	Original cartridge from ISMATEC characterized on a comparator, new cartridge modeled directly after original
Solar panel array	Blown away by wind	Loss of power to ISMA	Panels shaped like sails Panels not sufficiently anchored	None None	None None	Install wind shields on each panel Anchor panels to ground Aluminum wind shields installed Panels anchored with concrete block